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Bond Strength Evaluation of Universal Adhesives Applied in Self-Etch and Etch-and-Rinse Modus on Sound and High-Level Irradiated Dentin—A Controlled Laboratory Study

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Abstract: Background: Radiotherapy is an important part of the medical therapy concept treating tumors in the head and neck region; however, the impact on the field of dentistry could be immense. The objective of this study was to evaluate the microtensile bond strength (μTBS) of universal adhesives and to compare the results of different application modes (etch-and-rinse and self-etch) on irradiated and sound human dentin. Methods: Dentin specimen of 180 extracted human wisdom teeth were divided in two superordinate groups. Ninety teeth received either no irradiation (control group) or the high-level therapeutic dose of 60 Gy (test group) applied fractionally (2 Gy/day, 5 days/week, 6 weeks). Samples from each group were randomly divided into six subgroups assigned to three established universal adhesives (Futurabond® U, VOCO; AdheSE® Universal, Ivoclar Vivadent; Xeno® Select, Dentsply) in both conceivable application modes: etch-and-rinse and self-etch (n = 15). Bond strength was assessed with a universal testing machine immediately after application and light curing of the adhesive materials. Statistical analysis was performed using two-way ANOVA ($p<0.01$) and Tukey's test ($p<0.05$). Results: The effect of dentin irradiation and application mode for the adhesive restoration was found to be significant ($p<0.01$, ANOVA). In all groups, a decrease of μTBS could be observed when used on irradiated dentin. However, no significant influence of high level radiation on the bond strength of Futurabond® U and AdheSE® Universal in both modes could be evaluated ($p>0.05$, Tukey's test). Solely in the case of the adhesive system Xeno® Select, significantly reduced bond strength could be determined in self-etch mode after irradiation (group 60 Xse) compared to the control group (0 Xse) ($p<0.05$, Tukey´s test). Conclusions: Within the limitations of an in vitro study, some effects of high-level tumor irradiation on the bond strength of universal adhesives can be detected. However, these effects were strongly depending on the used material and application mode.

Keywords: adhesive dentistry; dentin bonding; microtensile bond strength; radiotherapy; irradiation; universal adhesive system

1. Introduction

Universal adhesive systems have been on the market since 2011 and have become well established in the daily use of the majority of practicing dentists for bonding numerous types of direct and indirect restorations in recent years [1,2]. They are characterized by the fact that they can be used in all well-studied conditioning modes: either in etch-and-rinse or self-etch-mode, as well as following the clinical procedure of selective-enamel-etching [3–7].

Regarding adhesive dentistry, a distinction could be made between adhesion to enamel and adhesion to dentin [8–10]. Enamel is one of the hardest structures in the human body and consists of hydroxyapatite crystals, which form enamel prisms. The enamel consists of 94-96% inorganic hydroxyapatite and is thus more highly mineralized than the adjacent dentin [1,11]. Conditioning the enamel with phosphoric acid increases the surface area and allows infiltration of adhesives, which subsequently form a bond to the enamel by light-curing [12,13]. Adhesion to dentin is more difficult because dentin is much less mineralized than enamel and consists of about 70% inorganic and 20% organic components (mainly collagen) [14,15]. Dentinal tubules run in the dentin, passing through the dentin in an S-shape from the pulp to the enamel-dentin-junction. The odontoblast processes are located in the tubules.

When these dentinal tubules are opened, dentin liquor leaks out due to the internal pulp pressure. The organic components and the smear layer formed during preparation prevent the micromechanical adhesion of the hydrophobic composite to the dentin [16,17]. To enable an adhesive bond to dentin, it should be demineralized by conditioning using acidified agents removing the smear layer, alternating the dentin surface and ultimately leading to exposure of the dentinal collagen fibers [18,19]. A primer with hydrophilic components enables infiltrating the collagen network and the dentin surface. The subsequently applied hydrophobic adhesive stabilizes the collagen and enables copolymerization and formation of resin-tags, which are essential for the adhesive bond between the surface of dentin and adhesive materials [10,20].

In these days, bonding systems usually are divided into two major groups depending on the type of smear layer dissolution. The etch-and-rinse adhesives use a 35-40% phosphoric acid gel to condition enamel and dentin [21]. In contrast, self-etching adhesives use acidic monomers to condition both dentin and enamel, making the additional phosphoric acid application redundant [22].

All universal adhesives contain a phosphate ester ($R\text{-}O\text{-}PO_3H_2$) as a functional monomer. A requirement for micromechanical retention of the restoration is the interlocking through the formation of a so-called "hybrid layer" [23,24]. This layer is formed by the infiltration of an uncovered collagen network and of the preconditioned and altered dentin surface including the formation of resin-tags [25]. It should be improved by additional chemical interactions between functional monomers and components of the mineralized hard tissues. The "adhesion-decalcification-concept" (ADC) plays a crucial part here, in which the functional monomer either decalcifies the hard tissue or forms an ionic bond with it [26,27]. The 10-methacryloyloxydecyl-dihydrogen-phosphate (10-MDP), which was first synthesized by KURARAY Co. (Chiyoda, Japan) in the early 1980s, is considered to be particularly applicable. 10-MDP is an amphiphilic monomer that has both a hydrophilic and a hydrophobic component. The hydrophobic methacrylate group enables chemical bonding to composites and the hydrophilic phosphate group enables chemical bonding to tooth structure through ionic bonds to the calcium of hydroxyapatite to form stable calcium-phosphate-salts [28,29]. Due to the long carbon chain structure, the monomer is quite hydrophobic, which has a positive effect on hydrolysis resistance and subsequently the durability [28,29].

In the treatment of head and neck tumors, radiotherapy has established as a commonly used therapy for affected patients [30–32]. However, this kind of treatment also has adverse effects in the field of dentistry, such as mucositis, hyposalivation, loss of taste, osteoradionecrosis and radiation caries [33–38]. The irradiation dose for the treatment of head and neck tumors is usually between 54-70 Gy and is fractionally applied over time [30,39].

Considering the development of carious lesions after irradiation, those usually appear about 4-6 months after treatment. The reasons for this are hyposalivation due to damage to the salivary glands and changes in the oral microbiome with an increase in cariogenic bacteria [36,40].

Furthermore, some studies have demonstrated an influence of therapeutic irradiation on the chemical and mechanical properties of dentin [41]. Looking at the structure of dentin, it has a higher fluid content than enamel, which results in a higher sensitivity to structural changes [42]. During irradiation, the water molecules located in the dentin tubules form reactive free radicals, such as oxygen and hydrogen peroxide. The interaction of these radicals with biomolecules of dentin can result in cell destruction [43] and denaturation of organic structures in dentin [44], which is dominated by collagen fibers resulting in a disconnection of the calcium from the collagen side chains.

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As a consequence, these changes to the collagen and dentin tubules may compromise the dentin bond by affecting the formation of the hybrid layer [45–47]. A reduction or loss of resin attachments and thin hybrid layers has been described in a previous investigation [48].

Therefore, the present study is intended to investigate the microtensile bond strength (μTBS) of universal adhesive systems on sound and highly irradiated dentin and, in particular, to evaluate the effect of different conditioning modes. The null hypothesis states that there is no difference in microtensile bond strength of the tested universal adhesives by using different conditioning modes on sound and irradiated dentin (control vs. test group).

2. Materials and Methods

2.1. Preparation of specimens

180 freshly extracted, caries-free human wisdom teeth were included in this study, which were stored in saline (0.9% NaCl, B. Braun Melsungen AG, Melsungen, Germany) at room temperature prior to the experimental procedure. The specimens were prepared from the extracted teeth to allow the continuous simulation of dentin perfusion and intrapulpal pressure [49]. The roots were separated from the crown and grounded using a grinding machine with constant water-cooling (RotoPol-35, Struers GmbH, Willich, Germany) until the access to the coronal pulp chamber was possible. The pulp tissue was removed and the specimens were then reduced from the occlusal surface until the distance between the prepared occlusal plateau and the coronal roof of the pulp chamber was set at 2.0 mm (±0.2 mm). The apical area was then parallelized to the occlusal plateau and reduced to a total specimen thickness of 4.0 mm (±0.2 mm) (Figure 1).

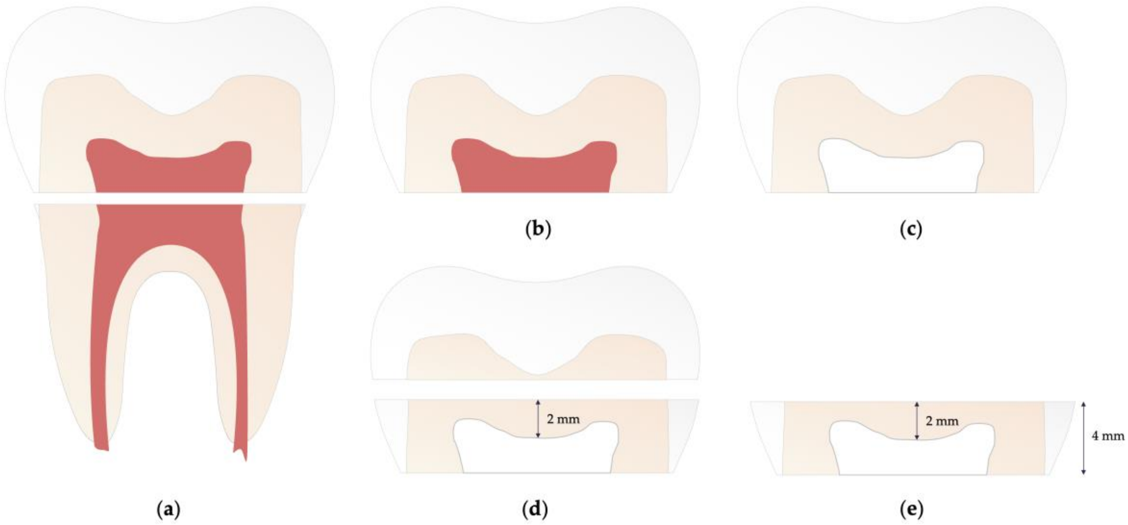

Figure 1. Schematic illustration of preparation of the dentin specimen: (a) Removal of the roots until the pulp chamber is exposed. (b) Coronal part of teeth with exposed pulp chamber. (c) Coronal part after removing the pulp tissue. (d) Grinding coronal part until a thickness of 2 mm above the pulp chamber roof was achieved. (e) Final dentin specimen with a total thickness of 4 mm and 2 mm coronal the pulp chamber roof.

2.2. Irradiation of specimens

These prepared specimens were stored in saline (0.9% NaCl) and randomly divided into two main groups. The first group (n=90) received no further treatment (control group), while the samples of the other group (n=90) were irradiated (test group). Radiation was delivered fractionally with a linear accelerator (ONCOR Impression IMRT Plus, Siemens AG, Munich, Germany) at doses of 2 Gy, 5 consecutive days per week for 6 weeks, according to conventional radiotherapy for head and neck cancer [41,50]. For homogeneity, specimens were always stored in saline, which was renewed daily [50], and placed in a single plane during radiation treatment (Figure 2).

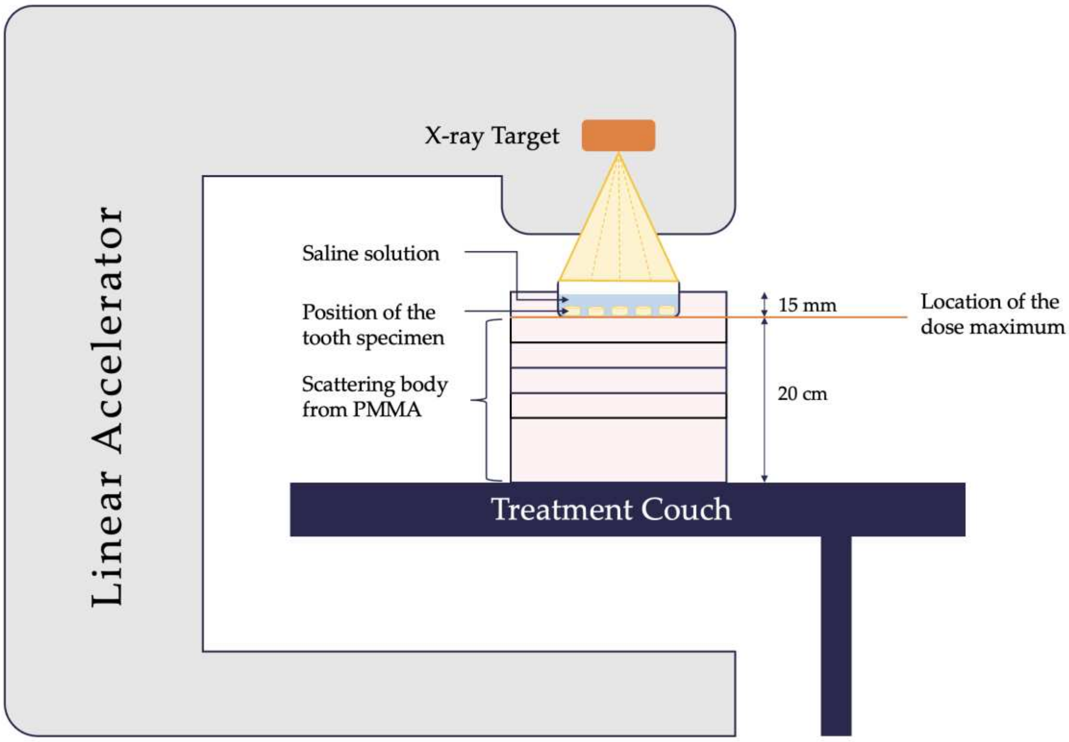

Figure 2. Schematic illustration of the set-up during irradiation of the specimen.

2.3. Materials used to perform the experimental procedure

The ninety specimens in each main group (non-irradiated control and irradiated test group) were randomly divided into six subgroups. The following three different universal bonding agents were tested in both self-etch and etch-and-rinse modes resulting in 12 subgroups of 15 samples each (Table 1).

Table 1. The twelve test groups assigned to three universal adhesives either without or with irradiation (n=15 samples).

	0 Gy (n=90)	**60 Gy (n=90)**
Futurabond® U	0 Fer	60 Fer
	0 Fse	60 Fse
AdheSE® Universal	0 Aer	60 Aer
	0 Ase	60 Ase
Xeno® Select	0 Xer	60 Xer
	0 Xse	60 Xse

The main components of the adhesives used (according to the safety data sheets of the particular manufacturers) are presented in Table 2.

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Table 2. Materials used in this study and their main components provided by the manufacturers.

Material	pH	Main components*	Manufacturer
Futurabond® U	2,3	BIS-GMA[1], HEMA[2], HEDMA[3], acidic adhesive monomer, UDMA[4], 10-MDP[5], catalyst	VOCO GmbH, Cuxhaven, Liechtenstein, Germany
AdheSE® Universal	2,5-3	BIS-GMA[1], HEMA[2], ethanol, 10-MDP[5], D3MA[6] , MCAP[7], camphorquinone	Ivoclar Vivadent AG, Schaan, Liechtenstein
Xeno® Select	< 2	bifunctional acrylates, tert-butyl alcohol, functionalized phosphoric acid ester (ethyl 2-[5-dihydrogen phosphoryl-5,2-dioxapentyl]acrylate), acidic acrylates, 4-dimethylaminobenzonitrile	Dentsply Sirona, Konstanz, Germany
Total Etch		37% Phosphoric Acid	Ivoclar Vivadent AG, Schaan, Liechtenstein
GrandioSO®, Color A2		Monomers: BIS-GMA[1], BIS-EMA[8], TEGDMA[9] Fillers: glass ceramic, functionalized SiO2[10] nanoparticles	VOCO GmbH, Cuxhaven, Germany

*According to the material safety data sheets provided by the manufacturers. [1] BIS-GMA: bisphenol A-glycidyl methacrylate. [2] HEMA: 2-hydroxyethyl methacrylate. [3] HEDMA: 1,6-hexanediylbismethacrylate. [4] UDMA: urethane-dimethacrylate. [5] 10-MDP: methacryloyloxydecyl dihydrogen phosphate. [6] D3MA: 1,10-decandiol dimethacrylate. [7] MCAP: methacrylated cyboxylic acid polymer. [8] BIS-EMA: ethoxylated bisphenol-A dimethacrylate. [9] TEGDMA: triethyleneglycol dimethacrylate. [10] SiO2: Silicon dioxide.

Etching of the etch-and-rinse groups was accomplished using 37% phosphoric acid (Total Etch, Ivoclar Vivadent AG, Schaan, Liechtenstein).

To ensure the comparability of the results, the same composite resin (Grandio®SO color A2, VOCO GmbH, Cuxhaven, Germany) was used in all test groups.

The experimental trials were conducted using an universal testing machine (Z005, Zwick GmbH & Co. KG, Ulm, Germany).

2.4. Experimental procedure

The samples in the etch-and-rinse groups were all prepared in the same manner: 37% phosphoric acid gel was applied to the dentin surface for 15 seconds, then rinsed with water and dried with oil-free compressed air.

For the experimental procedure, the specimens were attached to a special experimental device in an universal testing machine (Figure 3). Simulation of intrapulpal pressure and thereby dentin perfusion was achieved by generating pressure of 30 cm H_2O by saline solution, which was maintained throughout the testing period. The universal bonding agents were applied to both the etch-and-rinse as well as to the self-etch samples according to the manufacturers' recommendations, which did not differ for the three materials tested: A layer of adhesive was rubbed into the dentin surface for 20 seconds using a brush tip. Oil-free compressed air was then used to spread the adhesive and remove any solvents. The adhesive was subsequently light-cured for 10 seconds.

The composite filling material was applied through an application sleeve that only allowed a standardized area with a diameter of 1 mm as the bonding surface. The applied filling material was polymerized for 30 s with a high-power LED light unit (Celalux® 2, VOCO GmbH, Cuxhaven, Germany) at 900-2000 mW/cm^2.

Microtensile bond strength was determined with an universal testing machine 15 minutes after application and curing of the adhesive and composite materials. The specimens were loaded in tensile at a rate of 1 mm/min. The maximum force registered was divided by the standardized size of the bonding surface (diameter 1 mm = 0.79 mm^2) to obtain µTBS.

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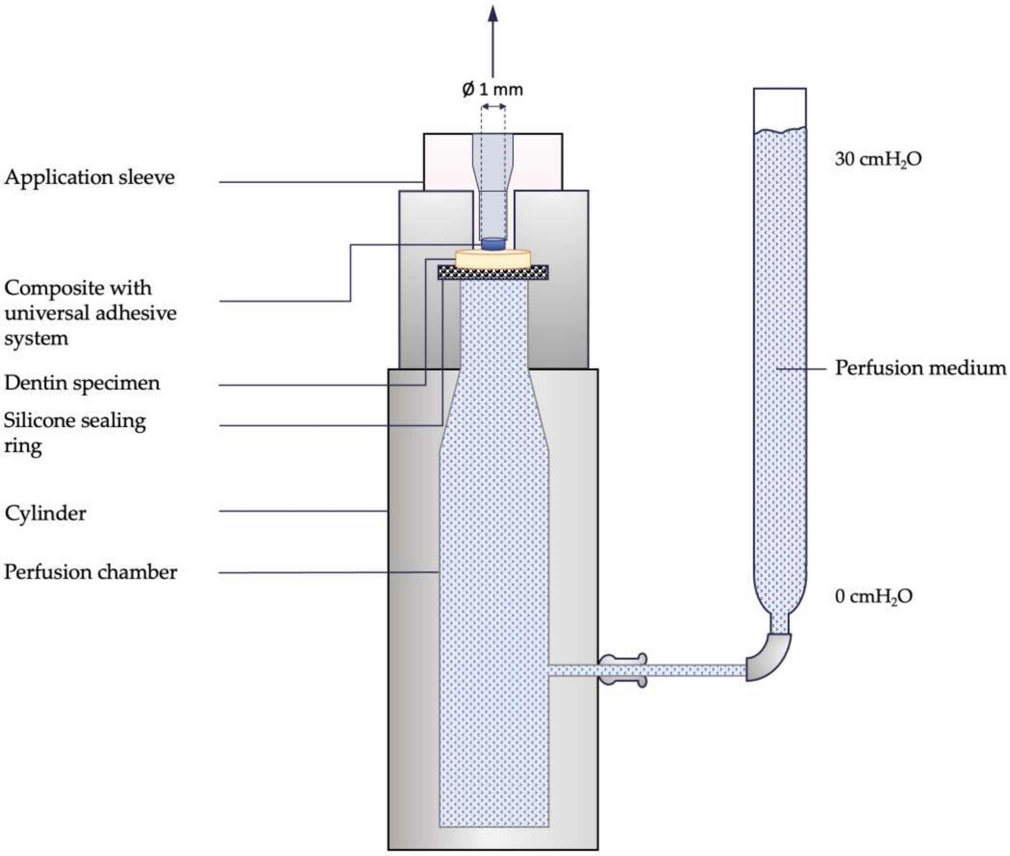

Figure 3. Schematic illustration of the used universal testing device allowing the application of the adhesive and the composite material on a standardized surface (Ø 1mm) under continuous dentin perfusion.

2.5. Data analysis and statistical evaluation

The mean microtensile bond strength and standard deviation were calculated for each experimental group. Statistical analysis was performed using SPSS® 25.0 (IBM®, Ehningen, Germany). Two-way ANOVA at a 1% significance level was used to evaluate differences between the results of all groups. Differences between the control and test groups were calculated using the Tukey's test at 5% significance level.

3. Results

The impact of irradiation on the adhesive tensile strength varied depending on the material used, but a reduction was evident in almost every experimental group. The influence of dentin irradiation and the applied conditioning mode for the adhesive restoration was found to be significant ($p<0.01$, ANOVA).

The results, in terms of mean microtensile bond strength and standard deviation of each group, are listed in Table 3.

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Table 3. Test results: mean microtensile bond strength in MPa (megapascal) and standard deviation.

Test group	0 Gy		60 Gy		Decrease of tensile bond strength after irradiation
F er	23,64	(± 6,76)	17,88	(± 7,54)	-24,4 %
F se	23,87	(± 7,49)	19,21	(± 7,34)	-19,5 %
A er	29,97	(± 7,18)	29,24	(± 7,28)	-2,4 %
A se	35,10	(± 8,41)	26,30	(± 10,07)	-25,1 %
X er	26,06	(± 8,20)	22,74	(± 6,22)	-12,7 %
X se	24,17	(± 8,36)	11,42	(± 3,86)	-52,8 %

The boxplot diagrams of Figures 4–6 depict the spread of the collected experimental data.

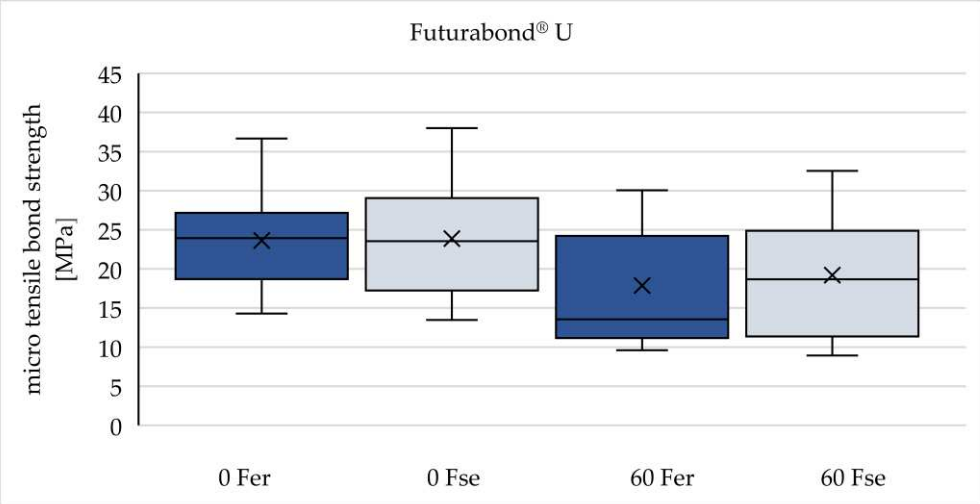

Figure 4. Boxplot of the microtensile bond strength of Futurabond® U. X = mean values in MPa.

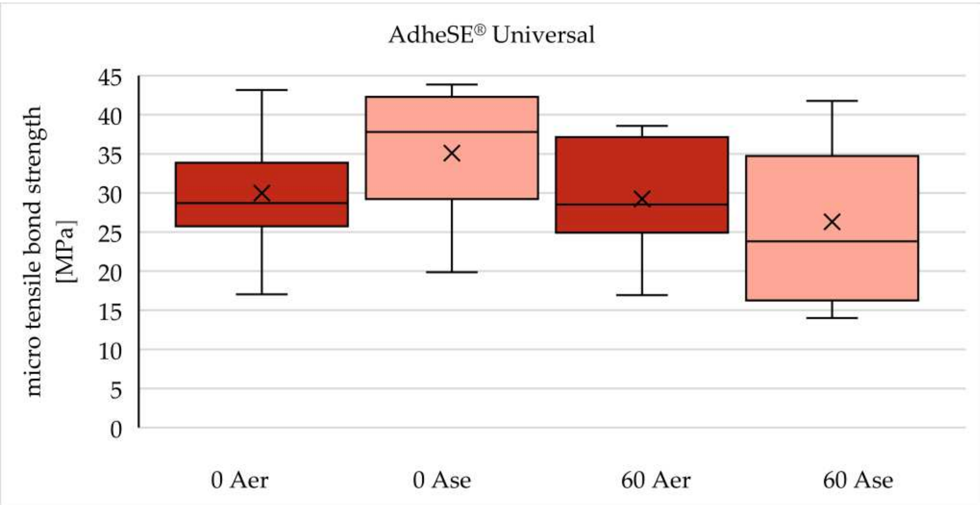

Figure 5. Boxplot of the microtensile bond strength of AdheSE® Universal. X = mean values in MPa.

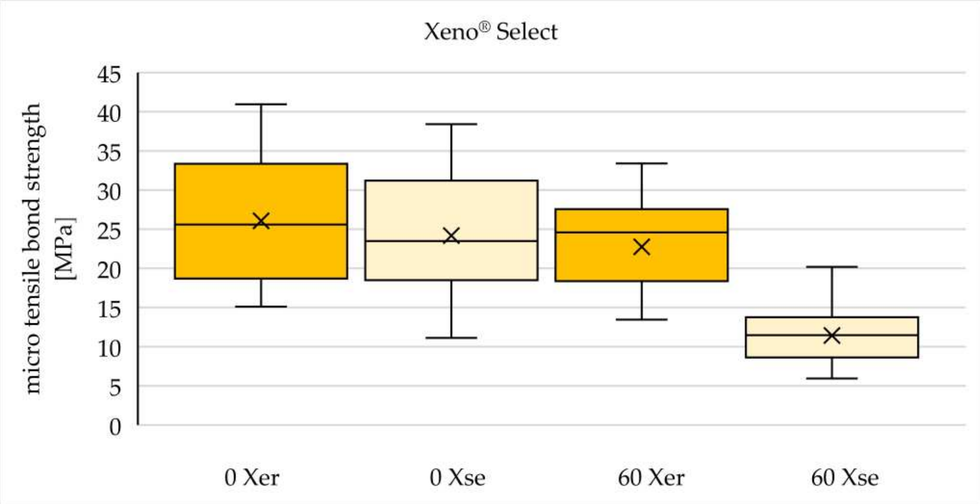

Figure 6. Boxplot of the microtensile bond strength of Xeno® Select. X = mean values in MPa.

Regarding the results for the material Futurabond® U, the value of the mean tensile bond strength without irradiation of etch-and-rinse- and self-etch-mode was almost the same (23.64 MPa (± 6.76 MPa) for etch-and rinse versus 23.87 MPa (± 7.49 MPa) for self-etch-mode). With 17.88 MPa (± 7.54 MPa) in etch-and-rinse mode and 19.21 MPa (± 7.34 MPa) in self-etch mode, a reduction of tensile bond strength was detectable after irradiation for both application modes of the material Futurabond® U, with a higher decrease in etch-and-rinse mode.

Unlike AdheSE® Universal: For this material microtensile bond strength without irradiation was higher overall with 29.97 MPa (± 7.18 MPa) in etch-and-rinse-mode and 35.10 MPa (± 8.41 MPa) in self-etch-mode. After irradiation, microtensile bond strength in etch-and-rinse-mode remained consistently high at 29.24 MPa (± 7.28 MPa). In self-etch-mode, a reduction of tensile bond strength of AdheSE® Universal to 26.30 MPa (± 10.07 MPa) after irradiation could be observed.

For the material Xeno® Select, reduction of the microtensile bond strength after irradiation was detected in both application modes. This decrease in etch-and-rinse mode from 26.06 MPa (± 8.20 MPa) to 22.74 MPa (± 6.22 MPa) was minor compared to the self-etch-mode with a decrease from 24.17 MPa (± 8.36 MPa) to 11.42 MPa (± 3.86 MPa), representing the major drop in tensile bond strength among all test groups.

A reduction of microtensile bond strength between non-irradiated and irradiated groups was observed in both groups. The extent of the decrease within a material and application mode group after irradiation is shown in Table 3. However, the influence of prior etching on the microtensile bond strength of Futurabond® U and AdheSE® Universal was not significant, neither on irradiated nor on non-irradiated dentin. Significantly reduced values of the microtensile bond strength in the self-etch mode compared to usage in etch-and-rinse mode when applied to irradiated dentin were only found in the test series of Xeno® Select ($p < 0.05$, Tukey test).

4. Discussion

In the present controlled laboratory study, the effect of the 2 conditioning modes etch-and-rinse and self-etch on the μTBS of irradiated and non-irradiated dentin was investigated. To prevent the influence of different operators, the preparation of the specimens, the application of the adhesive systems following exactly the recommendations of the manufacturer and the tensile bond strength tests were performed by an experienced dentist who had been trained in advance. The dentin specimens were also irradiated by an experienced radiation physicist to reduce the influence of individual variations in handling. Clinically caries-free wisdom teeth were selected to minimize effects of tooth substrate on bond strength. The dentin specimens were prepared approximately 2 mm above the pulp chamber to provide a comparable quantity and size of dentinal tubules.

In the present study, dentin specimens were irradiated with a total of 60 Gy, in 30 fractions of 2 Gy per day, 5 times a week for a period of 6 weeks, following conventional radiotherapy for the

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treatment of head-and-neck cancer [30,39]. During irradiation, all specimens were positioned in a plain level at the dose maximum. Although this does not correspond to the clinical circumstances, where the teeth are not necessarily located in the irradiation field, this experimental setup contributes to standardization. The experimental set-up with the generated pressure of 30 cmH_2O was intended to imitate the clinical conditions as closely as possible. As previously described, dentin liquor drains from the trimmed dentinal tubules due to the intrapulpal pressure [16]. This moist environment is challenging for adhesive bonding, and simulating intrinsic humidity; these test results have a higher relevance for the clinical situation despite in vitro conditions. The application sleeve with a cylindrical slot of 1 mm diameter ensured that each specimen obtained a standardized bonding surface of 0.79 mm^2 , which corresponds to the criteria for a microtensile bond strength test [51].

Comparing universal adhesives with other adhesive systems, they are characterized by their ability to be applied in different conditioning modes, as described above. The literature shows varying results regarding the influence of conditioning modes on the adhesive bond strength of different restorations [52–55]. Iatrogenic processing of dentin creates the so-called "smear layer", which is a physical barrier to the infiltration of the adhesive system [56,57]. Pretreatment with acidic agents, such as phosphoric acid or acidic monomers, can remove this layer and allow infiltration into the exposed collagen network [57,58]. Universal adhesive systems have acidic monomers that allow demineralization at the dentin surface, but at the same time form a chemical bond with the calcium ions of hydroxyapatite, forming stable calcium-salt complexes [1,2]. In their study, Yamauchi et al. [55] compared 3 modern universal adhesives in terms of shear bond strength (SBS) and shear fatigue resistance (SFR) in both application modes. One adhesive system revealed higher SBS and SFR for phosphoric acid etching compared to the self-etch mode application. The other investigated adhesive systems did not show significant differences between the conditioning modes. Similar results were confirmed by other studies [53,54].

Furthermore, like in the present study, in the above mentioned investigations differences in bond strength were described when comparing different materials from different manufacturers. The pH of the applied adhesive systems plays a role here: ultra-mild universal adhesive systems showed an advantage for the additional use of phosphoric acid [52]. Presumably, the acidity of these products is not sufficient to expose enough collagen fibers to ensure good hybridization. Brkanovic et. al. [52] using adhesive systems with a low pH revealed increased adhesion in the self-etch mode compared to the etch-and-rinse mode measured by µTBS on dentin. The used materials in the present investigation showed nearly no differences in pH, so this factor could be neglected.

Modern universal adhesives differ in their components and especially in their functional monomers. A particularly well-known representative of this group is 10-MDP, which is characterized by its amphiphilic properties and the high activity of its two functional groups [59]. It forms stable collagen-phosphate complexes with the collagen present in dentin. In addition, the formed MDP-Ca salts are deposited on the collagen fibers and protect them from degradation by proteases and collagenases [60]. These collagenases include the well-known matrix metalloproteinases, whose negative influence on the adhesive bond through nanoleakage has been described in many publications [2,61–63]. In our study, Futurabond® U and AdheSE® Universal contain 10-MDP as a functional monomer [64,65], for which no significant difference between the etch-and-rinse and self-etch groups could be observed with regard to µTBS in irradiated and non-irradiated group.

Previous studies have described morphologic changes in dentin and enamel as a result of irradiation [66–68]. Structural changes in dentin, such as obliteration of dentin tubules [69,70], dissolution of the collagen network, and ruptures in the dentin structure [70,71], as well as a reduction in microhardness [41,72], have been described as a result of irradiation. Other publications have shown weakening of the dentinoenamel junction (DEJ) [67,72], which is mainly stabilized by a collagen network. As a result of irradiation, the collagen network is partially destroyed [70], resulting in a weak bond strength between enamel and dentin. These factors may be expressed in decreased bond strength of adhesive systems, which is consistent with the results of our study.

A possible mechanism for the change in structure of irradiated dentin could be the result of an oxidation reaction caused by irradiation. As described previously, radiation breaks down the water molecules in dentin with the release of reactive oxygen radicals [44]. This proteolytic degradation may affect collagenous and non-collagenous proteins in dentin structures [44]. Activation of MMP-20, which has the ability to cleave type IV collagen, has also been demonstrated, particularly in enamel and at the DEJ [73–75]. As a result, increased protein degradation may lead to deterioration of the physical properties of enamel and dentin [68,73,74].

It was only considered in this study that resin fillings were applied post-radiation, not the scenario where a patient might have fillings before radiotherapy starts. Irradiating an existing dental restoration might have distinct impacts on the bond strength, as determined by Arid et al. [76] in a prior study, or on the repair's long-term durability. Further investigation is necessary, especially concerning the recommendation to rehabilitate patients' dentition prior to radiotherapy [67]

Currently, there are only a limited number of studies that investigated the bond strength of adhesively bonded restorations on irradiated enamel or dentin and their clinical performance. Moreover, the studies showed different, partly contradictory results: Some of them did not find any influence of irradiation on the dentin bond strength [77–79], while other studies reported a reduction of the bond strength [45,76,80–82]. Naves et al. [83] also investigated μTBS on irradiated teeth, but they evaluated enamel bond strength in addition to dentin. Furthermore, they used a classic etch-and-rinse adhesive as opposed to the universal adhesive used in our study. The study showed that the bond strength on enamel after irradiation was superior to that on dentin, but in general had a negative effect on the microtensile bond strength values [83].

5. Conclusions

Despite the limitations of this laboratory study, a reduction of bond strength to irradiated dentin of universal adhesive systems was observed. However, the type of material and conditioning mode can affect bond strength to irradiated teeth. The 10-MDP-containing universal adhesives Futurabond® U and AdheSE® Universal, showed no significant difference in bond strength as measured by μTBS in both etch-and-rinse and self-etch modes. Nevertheless, acid etching with phosphoric acid seems to be advantageous for the use of ultra-mild adhesive systems like Xeno® Select.

Nevertheless, no firm recommendation can be made for the usage of universal adhesives in patients undergoing radiotherapy for head-and-neck cancer. On the one hand, the literature is still ambiguous in this regard, and on the other hand, further clinical investigations and long-term studies are desirable in order to establish a clinical guideline so that patients undergoing radiotherapy can receive the best possible dental treatment.

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Conflicts of Interest: The authors declare no conflict of interest. The manufacturers, providing the materials did not influence the design of the study; the data collection, the analysis, the interpretation of data; the writing and editing of the manuscript. They were not involved in the final decision to publish the results in polymers.

References

1. Sofan, E.; Sofan, A.; Palaia, G.; Tenore, G.; Romeo, U.; Migliau, G. Classification review of dental adhesive systems: from the IV generation to the universal type. *Ann Stomatol (Roma)* **2017**, *8*, 1-17, doi:10.11138/ads/2017.8.1.001.
2. Hardan, L.; Bourgi, R.; Kharouf, N.; Mancino, D.; Zarow, M.; Jakubowicz, N.; Haikel, Y.; Cuevas-Suárez, C.E. Bond Strength of Universal Adhesives to Dentin: A Systematic Review and Meta-Analysis. *Polymers (Basel)* **2021**, *13*, doi:10.3390/polym13050814.
3. Diniz, A.C.; Bandeca, M.C.; Pinheiro, L.M.; Dos Santosh Almeida, L.J., Jr.; Torres, C.R.; Borges, A.H.; Pinto, S.C.; Tonetto, M.R.; De Jesus Tavarez, R.R.; Firoozmand, L.M. Influence of Different Etching Modes on Bond Strength to Enamel using Universal Adhesive Systems. *J Contemp Dent Pract* **2016**, *17*, 820-825, doi:10.5005/jp-journals-10024-1937.
4. Follak, A.C.; Ilha, B.D.; Oling, J.; Savian, T.; Rocha, R.O.; Soares, F.Z.M. Clinical behavior of universal adhesives in non-carious cervical lesions: A randomized clinical trial. *J Dent* **2021**, *113*, 103747, doi:10.1016/j.jdent.2021.103747.
5. Josic, U.; Maravic, T.; Mazzitelli, C.; Radovic, I.; Jacimovic, J.; Del Bianco, F.; Florenzano, F.; Breschi, L.; Mazzoni, A. Is clinical behavior of composite restorations placed in non-carious cervical lesions influenced by the application mode of universal adhesives? A systematic review and meta-analysis. *Dent Mater* **2021**, *37*, e503-e521, doi:10.1016/j.dental.2021.08.017.
6. Josic, U.; Mazzitelli, C.; Maravic, T.; Radovic, I.; Jacimovic, J.; Mancuso, E.; Florenzano, F.; Breschi, L.; Mazzoni, A. The influence of selective enamel etch and self-etch mode of universal adhesives' application on clinical behavior of composite restorations placed on non-carious cervical lesions: A systematic review and meta-analysis. *Dent Mater* **2022**, *38*, 472-488, doi:10.1016/j.dental.2022.01.002.
7. Gernhardt, C.R.; Nguyen, A.D.; Michaelis, M.; Pütz, N. Clinical Outcome of Class I and II Restorations with and without an Intermediary Layer of a Flowable Composite after 24 Months: A Prospective, Randomized, Split-Mouth-Designed, Controlled and Single-Blinded Clinical Trial. *Applied Sciences* **2023**, *13*, 4224.
8. Wiegand, A.; Lechte, C.; Kanzow, P. Adhesion to eroded enamel and dentin: systematic review and meta-analysis. *Dent Mater* **2021**, *37*, 1845-1853, doi:10.1016/j.dental.2021.09.014.
9. Cruz, J.; Sousa, B.; Coito, C.; Lopes, M.; Vargas, M.; Cavalheiro, A. Microtensile bond strength to dentin and enamel of self-etch vs. etch-and-rinse modes of universal adhesives. *Am J Dent* **2019**, *32*, 174-182.
10. Jacker-Guhr, S.; Sander, J.; Luehrs, A.K. How "Universal" is Adhesion? Shear Bond Strength of Multi-mode Adhesives to Enamel and Dentin. *J Adhes Dent* **2019**, *21*, 87-95, doi:10.3290/j.jad.a41974.
11. Jayasudha; Baswaraj; H, K.N.; K, B.P. Enamel regeneration - current progress and challenges. *J Clin Diagn Res* **2014**, *8*, Ze06-09, doi:10.7860/jcdr/2014/10231.4883.
12. Devarasa, G.M.; Subba Reddy, V.V.; Chaitra, N.L.; Swarna, Y.M. Self-etching adhesive on intact enamel, with and without pre-etching. *Microsc Res Tech* **2012**, *75*, 650-654, doi:10.1002/jemt.21106.
13. Hajizadeh, H.; Nemati-Karimooy, A.; Nasseh, A.; Rahmanpour, N. Evaluating the shear bond strength of enamel and dentin with or without etching: A comparative study between dimethacrylate-based and silorane-based adhesives. *J Clin Exp Dent* **2015**, *7*, e563-568, doi:10.4317/jced.52322.
14. Chen, L.; Al-Bayatee, S.; Khurshid, Z.; Shavandi, A.; Brunton, P.; Ratnayake, J. Hydroxyapatite in Oral Care Products-A Review. *Materials (Basel)* **2021**, *14*, doi:10.3390/ma14174865.
15. Goldberg, M.; Kulkarni, A.B.; Young, M.; Boskey, A. Dentin: structure, composition and mineralization. *Front Biosci (Elite Ed)* **2011**, *3*, 711-735, doi:10.2741/e281.
16. Siriporananon, C.; Senawongse, P.; Sattabanasuk, V.; Srimaneekarn, N.; Sano, H.; Saikaew, P. Effects of dentin surface preparations on bonding of self-etching adhesives under simulated pulpal pressure. *Restor Dent Endod* **2022**, *47*, e4, doi:10.5395/rde.2022.47.e4.
17. Alshaikh, K.H.; Hamama, H.H.H.; Mahmoud, S.H. Effect of smear layer deproteinization on bonding of self-etch adhesives to dentin: a systematic review and meta-analysis. *Restor Dent Endod* **2018**, *43*, e14, doi:10.5395/rde.2018.43.e14.
18. Scheffel, D.L.; Tenuta, L.M.; Cury, J.A.; Hebling, J. Effect of acid etching time on demineralization of primary and permanent coronal dentin. *Am J Dent* **2012**, *25*, 235-238.
19. Perdigão, J. Dentin bonding-variables related to the clinical situation and the substrate treatment. *Dent Mater* **2010**, *26*, e24-37, doi:10.1016/j.dental.2009.11.149.

20. Katsuki, S.; Takamizawa, T.; Yokoyama, M.; Sai, K.; Tamura, T.; Ishii, R.; Kamimoto, A.; Miyazaki, M. Influence of bonding agent application method on the dentin bond durability of a two-step adhesive utilizing a universal-adhesive-derived primer. *Eur J Oral Sci* **2022**, *130*, e12868, doi:10.1111/eos.12868.
21. Pashley, D.H.; Tay, F.R.; Breschi, L.; Tjäderhane, L.; Carvalho, R.M.; Carrilho, M.; Tezvergil-Mutluay, A. State of the art etch-and-rinse adhesives. *Dent Mater* **2011**, *27*, 1-16, doi:10.1016/j.dental.2010.10.016.
22. Masarwa, N.; Mohamed, A.; Abou-Rabii, I.; Abu Zaghlan, R.; Steier, L. Longevity of Self-etch Dentin Bonding Adhesives Compared to Etch-and-rinse Dentin Bonding Adhesives: A Systematic Review. *J Evid Based Dent Pract* **2016**, *16*, 96-106, doi:10.1016/j.jebdp.2016.03.003.
23. Breschi, L.; Maravic, T.; Cunha, S.R.; Comba, A.; Cadenaro, M.; Tjäderhane, L.; Pashley, D.H.; Tay, F.R.; Mazzoni, A. Dentin bonding systems: From dentin collagen structure to bond preservation and clinical applications. *Dental Materials* **2018**, *34*, 78-96.
24. Frassetto, A.; Breschi, L.; Turco, G.; Marchesi, G.; Di Lenarda, R.; Tay, F.R.; Pashley, D.H.; Cadenaro, M. Mechanisms of degradation of the hybrid layer in adhesive dentistry and therapeutic agents to improve bond durability—A literature review. *Dental Materials* **2016**, *32*, e41-e53.
25. Stape, T.H.S.; Mutluay, M.M.; Tjäderhane, L.; Uurasjärvi, E.; Koistinen, A.; Tezvergil-Mutluay, A. The pursuit of resin-dentin bond durability: Simultaneous enhancement of collagen structure and polymer network formation in hybrid layers. *Dental Materials* **2021**, *37*, 1083-1095, doi:10.1016/j.dental.2021.03.010.
26. Van Meerbeek, B.; Yoshihara, K.; Van Landuyt, K.; Yoshida, Y.; Peumans, M. From Buonocore's Pioneering Acid-Etch Technique to Self-Adhering Restoratives. A Status Perspective of Rapidly Advancing Dental Adhesive Technology. *J Adhes Dent* **2020**, *22*, 7-34, doi:10.3290/j.jad.a43994.
27. Van Meerbeek, B.; Yoshihara, K.; Yoshida, Y.; Mine, A.; De Munck, J.; Van Landuyt, K.L. State of the art of self-etch adhesives. *Dent Mater* **2011**, *27*, 17-28, doi:10.1016/j.dental.2010.10.023.
28. Carrilho, E.; Cardoso, M.; Marques Ferreira, M.; Marto, C.M.; Paula, A.; Coelho, A.S. 10-MDP Based Dental Adhesives: Adhesive Interface Characterization and Adhesive Stability-A Systematic Review. *Materials (Basel)* **2019**, *12*, doi:10.3390/ma12050790.
29. Fehrenbach, J.; Isolan, C.P.; Münchow, E.A. Is the presence of 10-MDP associated to higher bonding performance for self-etching adhesive systems? A meta-analysis of in vitro studies. *Dent Mater* **2021**, *37*, 1463-1485, doi:10.1016/j.dental.2021.08.014.
30. Alterio, D.; Marvaso, G.; Ferrari, A.; Volpe, S.; Orecchia, R.; Jereczek-Fossa, B.A. Modern radiotherapy for head and neck cancer. *Semin Oncol* **2019**, *46*, 233-245, doi:10.1053/j.seminoncol.2019.07.002.
31. Caudell, J.J.; Torres-Roca, J.F.; Gillies, R.J.; Enderling, H.; Kim, S.; Rishi, A.; Moros, E.G.; Harrison, L.B. The future of personalised radiotherapy for head and neck cancer. *Lancet Oncol* **2017**, *18*, e266-e273, doi:10.1016/s1470-2045(17)30252-8.
32. Brennan, P.A.; Bradley, K.L.; Brands, M. Intensity-modulated radiotherapy in head and neck cancer - an update for oral and maxillofacial surgeons. *Br J Oral Maxillofac Surg* **2017**, *55*, 770-774, doi:10.1016/j.bjoms.2017.07.019.
33. Sonis, S.T. Oral mucositis in head and neck cancer: risk, biology, and management. *Am Soc Clin Oncol Educ Book* **2013**, doi:10.1200/EdBook_AM.2013.33.e236.
34. Brook, I. Early side effects of radiation treatment for head and neck cancer. *Cancer Radiother* **2021**, *25*, 507-513, doi:10.1016/j.canrad.2021.02.001.
35. Schulz, R.E.; Bonzanini, L.I.L.; Ortigara, G.B.; Soldera, E.B.; Danesi, C.C.; Antoniazzi, R.P.; Ferrazzo, K.L. Prevalence of hyposalivation and associated factors in survivors of head and neck cancer treated with radiotherapy. *J Appl Oral Sci* **2021**, *29*, e20200854, doi:10.1590/1678-7757-2020-0854.
36. Moore, C.; McLister, C.; Cardwell, C.; O'Neill, C.; Donnelly, M.; McKenna, G. Dental caries following radiotherapy for head and neck cancer: A systematic review. *Oral Oncol* **2020**, *100*, 104484, doi:10.1016/j.oraloncology.2019.104484.
37. Sroussi, H.Y.; Epstein, J.B.; Bensadoun, R.J.; Saunders, D.P.; Lalla, R.V.; Migliorati, C.A.; Heaivilin, N.; Zumsteg, Z.S. Common oral complications of head and neck cancer radiation therapy: mucositis, infections, saliva change, fibrosis, sensory dysfunctions, dental caries, periodontal disease, and osteoradionecrosis. *Cancer Med* **2017**, *6*, 2918-2931, doi:10.1002/cam4.1221.
38. Kuhnt, T.; Jirsak, N.; Müller, A.C.; Pelz, T.; Gernhardt, C.; Schaller, H.G.; Janich, M.; Gerlach, R.; Dunst, J. [Quantitative and qualitative investigations of salivary gland function in dependence on irradiation dose and volume for reduction of xerostomia in patients with head-and-neck cancer]. *Strahlenther Onkol* **2005**, *181*, 520-528, doi:10.1007/s00066-005-1366-y.

39. Corvò, R. Evidence-based radiation oncology in head and neck squamous cell carcinoma. *Radiother Oncol* **2007**, *85*, 156-170, doi:10.1016/j.radonc.2007.04.002.
40. Hey, J.; Seidel, J.; Schweyen, R.; Paelecke-Habermann, Y.; Vordermark, D.; Gernhardt, C.; Kuhnt, T. The influence of parotid gland sparing on radiation damages of dental hard tissues. *Clin Oral Investig* **2013**, *17*, 1619-1625, doi:10.1007/s00784-012-0854-6.
41. Gonçalves, L.M.; Palma-Dibb, R.G.; Paula-Silva, F.W.; Oliveira, H.F.; Nelson-Filho, P.; Silva, L.A.; Queiroz, A.M. Radiation therapy alters microhardness and microstructure of enamel and dentin of permanent human teeth. *J Dent* **2014**, *42*, 986-992, doi:10.1016/j.jdent.2014.05.011.
42. Douchy, L.; Gauthier, R.; Abouelleil-Sayed, H.; Colon, P.; Grosgogeat, B.; Bosco, J. The effect of therapeutic radiation on dental enamel and dentin: A systematic review. *Dental Materials* **2022**, *38*, e181-e201, doi:10.1016/j.dental.2022.04.014.
43. Fang, Y.-Z.; Yang, S.; Wu, G. Free radicals, antioxidants, and nutrition. *Nutrition* **2002**, *18*, 872-879.
44. Pioch, T.; Golfels, D.; Staehle, H.J. An experimental study of the stability of irradiated teeth in the region of the dentinoenamel junction. *Endod Dent Traumatol* **1992**, *8*, 241-244, doi:10.1111/j.1600-9657.1992.tb00251.x.
45. Mellara, T.d.S.; Paula-Silva, F.W.G.; Arid, J.; de Oliveira, H.F.; Nelson-Filho, P.; Bezerra Silva, R.A.; Torres, F.M.; Faraoni, J.J.; Palma-Dibb, R.G.; de Queiroz, A.M. Radiotherapy impairs adhesive bonding in primary teeth: An in vitro study. *Journal of Dentistry for Children* **2020**, *87*, 69-76.
46. Oglakci, B. How does radiotherapy affect the adhesion of universal adhesive to enamel and dentin? A qualitative and quantitative analysis?. Odovtos [online]. 2022, vol. 24, n. 3. **2022**, *24*, 2215-3411, doi:10.15517/ijds.2022.50665
47. Broscheit, S.; Vordermark, D.; Gerlach, R.; Gernhardt, C.R. Impact of Preceded Tumor Therapeutic Irradiation on the Microtensile Bond Strength of Universal Adhesives Applied in Self-Etch Mode to Human Dentin In Vitro. *Applied Sciences* **2023**, *13*, 7873.
48. Eggmann, F.; Hwang, J.D.; Ayub, J.M.; Mante, F.K. Impact of Irradiation on the Adhesive Performance of Resin-Based Dental Biomaterials: A Systematic Review of Laboratory Studies. *Materials (Basel)* **2023**, *16*, doi:10.3390/ma16072580.
49. Gernhardt, C.R.; Schaller, H.G.; Kielbassa, A.M. The influence of human plasma used for dentin perfusion on tensile bond strength of different light-curing materials. *Am J Dent* **2005**, *18*, 318-322.
50. Kielbassa, A.M.; Beetz, I.; Schendera, A.; Hellwig, E. Irradiation effects on microhardness of fluoridated and non-fluoridated bovine dentin. *Eur J Oral Sci* **1997**, *105*, 444-447, doi:10.1111/j.1600-0722.1997.tb02142.x.
51. Sano, H.; Shono, T.; Sonoda, H.; Takatsu, T.; Ciucchi, B.; Carvalho, R.; Pashley, D.H. Relationship between surface area for adhesion and tensile bond strength--evaluation of a micro-tensile bond test. *Dent Mater* **1994**, *10*, 236-240, doi:10.1016/0109-5641(94)90067-1.
52. Brkanović, S.; Sever, E.K.; Vukelja, J.; Ivica, A.; Miletić, I.; Krmek, S.J. Comparison of Different Universal Adhesive Systems on Dentin Bond Strength. *Materials (Basel)* **2023**, *16*, doi:10.3390/ma16041530.
53. Rosa, W.L.d.O.d.; Piva, E.; Silva, A.F.d. Bond strength of universal adhesives: A systematic review and meta-analysis. *Journal of Dentistry* **2015**, *43*, 765-776, doi:10.1016/j.jdent.2015.04.003.
54. Takamizawa, T.; Barkmeier, W.W.; Tsujimoto, A.; Berry, T.P.; Watanabe, H.; Erickson, R.L.; Latta, M.A.; Miyazaki, M. Influence of different etching modes on bond strength and fatigue strength to dentin using universal adhesive systems. *Dent Mater* **2016**, *32*, e9-21, doi:10.1016/j.dental.2015.11.005.
55. Yamauchi, K.; Tsujimoto, A.; Jurado, C.A.; Shimatani, Y.; Nagura, Y.; Takamizawa, T.; Barkmeier, W.W.; Latta, M.A.; Miyazaki, M. Etch-and-rinse vs self-etch mode for dentin bonding effectiveness of universal adhesives. *J Oral Sci* **2019**, *61*, 549-553, doi:10.2334/josnusd.18-0433.
56. Chowdhury, A.; Islam, R.; Alam, A.; Matsumoto, M.; Yamauti, M.; Carvalho, R.M.; Sano, H. Variable Smear Layer and Adhesive Application: The Pursuit of Clinical Relevance in Bond Strength Testing. *Int J Mol Sci* **2019**, *20*, doi:10.3390/ijms20215381.
57. Kambara, K.; Nakajima, M.; Hosaka, K.; Takahashi, M.; Thanatvarakorn, O.; Ichinose, S.; Foxton, R.M.; Tagami, J. Effect of smear layer treatment on dentin bond of self-adhesive cements. *Dent Mater J* **2012**, *31*, 980-987, doi:10.4012/dmj.2012-031.
58. Saikaew, P.; Sattabanasuk, V.; Harnirattisai, C.; Chowdhury, A.; Carvalho, R.; Sano, H. Role of the smear layer in adhesive dentistry and the clinical applications to improve bonding performance. *Jpn Dent Sci Rev* **2022**, *58*, 59-66, doi:10.1016/j.jdsr.2021.12.001.

59. Yoshida, Y.; Nagakane, K.; Fukuda, R.; Nakayama, Y.; Okazaki, M.; Shintani, H.; Inoue, S.; Tagawa, Y.; Suzuki, K.; De Munck, J.; et al. Comparative study on adhesive performance of functional monomers. *J Dent Res* **2004**, *83*, 454-458, doi:10.1177/154405910408300604.
60. Jin, X.; Han, F.; Wang, Q.; Yuan, X.; Zhou, Q.; Xie, H.; Niu, L.; Chen, C. The roles of 10-methacryloyloxydecyl dihydrogen phosphate and its calcium salt in preserving the adhesive–dentin hybrid layer. *Dental Materials* **2022**, *38*, 1194-1205, doi:10.1016/j.dental.2022.06.007.
61. Fronza, B.M.; Braga, R.R.; Cadenaro, M. Dental Adhesives-Surface Modifications of Dentin Structure for Stable Bonding. *Dent Clin North Am* **2022**, *66*, 503-515, doi:10.1016/j.cden.2022.05.002.
62. Kiuru, O.; Sinervo, J.; Vähänikkilä, H.; Anttonen, V.; Tjäderhane, L. MMP Inhibitors and Dentin Bonding: Systematic Review and Meta-Analysis. *Int J Dent* **2021**, *2021*, 9949699, doi:10.1155/2021/9949699.
63. Montagner, A.F.; Sarkis-Onofre, R.; Pereira-Cenci, T.; Cenci, M.S. MMP Inhibitors on Dentin Stability: A Systematic Review and Meta-analysis. *J Dent Res* **2014**, *93*, 733-743, doi:10.1177/0022034514538046.
64. Tian, F.; Zhou, L.; Zhang, Z.; Niu, L.; Zhang, L.; Chen, C.; Zhou, J.; Yang, H.; Wang, X.; Fu, B.; et al. Paucity of Nanolayering in Resin-Dentin Interfaces of MDP-based Adhesives. *J Dent Res* **2016**, *95*, 380-387, doi:10.1177/0022034515623741.
65. Torres, C.R.; Zanatta, R.F.; Silva, T.J.; Huhtala, M.F.; Borges, A.B. Influence of previous acid etching on bond strength of universal adhesives to enamel and dentin. *Gen Dent* **2017**, *65*, e17-e21.
66. Grötz, K.A.; Duschner, H.; Kutzner, J.; Thelen, M.; Wagner, W. [Histotomography studies of direct radiogenic dental enamel changes]. *Mund Kiefer Gesichtschir* **1998**, *2*, 85-90, doi:10.1007/s100060050035.
67. Kielbassa, A.M.; Hinkelbein, W.; Hellwig, E.; Meyer-Lückel, H. Radiation-related damage to dentition. *The Lancet Oncology* **2006**, *7*, 326-335, doi:10.1016/S1470-2045(06)70658-1.
68. Lieshout, H.F.J.; Bots, C.P. The effect of radiotherapy on dental hard tissue—a systematic review. *Clinical Oral Investigations* **2014**, *18*, 17-24, doi:10.1007/s00784-013-1034-z.
69. Martini, G.R.; Bortoluzzi, E.A.; Minamisako, M.C.; Bordignon, N.C.T.; Rodrigues, P.M.; Gondak, R. Impact of radiotherapy on the morphological and compositional structure of intra-radicular dentin. *Braz Dent J* **2023**, *34*, 45-51, doi:10.1590/0103-6440202305101.
70. Al-Nawas, B.; Grötz, K.; Rose, E.; Duschner, H.; Kann, P.; Wagner, W. Using ultrasound transmission velocity to analyse the mechanical properties of teeth after in vitro, in situ, and in vivo irradiation. *Clinical oral investigations* **2000**, *4*, 168-172.
71. Silva, A.; Alves, F.; Antunes, A.; Goes, M.; Lopes, M. Patterns of demineralization and dentin reactions in radiation-related caries. *Caries research* **2009**, *43*, 43-49.
72. Siripamitdul, P.; Sivavong, P.; Osathanon, T.; Pianmee, C.; Sangsawatpong, W.; Bunsong, C.; Nantanapiboon, D. The Effects of Radiotherapy on Microhardness and Mineral Composition of Tooth Structures. *Eur J Dent* **2023**, *17*, 357-364, doi:10.1055/s-0042-1746414.
73. McGuire, J.D.; Gorski, J.P.; Dusevich, V.; Wang, Y.; Walker, M.P. Type IV collagen is a novel DEJ biomarker that is reduced by radiotherapy. *J Dent Res* **2014**, *93*, 1028-1034, doi:10.1177/0022034514548221.
74. McGuire, J.D.; Mousa, A.A.; Zhang, B.J.; Todoki, L.S.; Huffman, N.T.; Chandrababu, K.B.; Moradian-Oldak, J.; Keightley, A.; Wang, Y.; Walker, M.P.; et al. Extracts of irradiated mature human tooth crowns contain MMP-20 protein and activity. *J Dent* **2014**, *42*, 626-635, doi:10.1016/j.jdent.2014.02.013.
75. Lu, Y.; Papagerakis, P.; Yamakoshi, Y.; Hu, J.C.; Bartlett, J.D.; Simmer, J.P. Functions of KLK4 and MMP-20 in dental enamel formation. *Biol Chem* **2008**, *389*, 695-700, doi:10.1515/bc.2008.080.
76. Arid, J.; Palma-Dibb, R.G.; de Oliveira, H.F.; Nelson-Filho, P.; de Carvalho, F.K.; da Silva, L.A.B.; de Siqueira Mellara, T.; da Silva, R.A.B.; Faraoni, J.J.; de Queiroz, A.M. Radiotherapy impairs adhesive bonding in permanent teeth. *Support Care Cancer* **2020**, *28*, 239-247, doi:10.1007/s00520-019-04782-5.
77. Galetti, R.; Santos-Silva, A.R.; Nogueira da Gama Antunes, A.; de Abreu Alves, F.; Lopes, M.A.; de Goes, M.F. Radiotherapy does not impair dentin adhesive properties in head and neck cancer patients. *Clinical Oral Investigations* **2014**, *18*, 1771-1778, doi:10.1007/s00784-013-1155-4.
78. de Barros da Cunha, S.R.; Minorin Mendes Ramos, P.A.; Kalil Haddad, C.M.; Fernandes da Silva, J.L.; Fregnani, E.R.; Corrêa Aranha, A.C. Effects of Different Radiation Doses on the Bond Strengths of Two Different Adhesive Systems to Enamel and Dentin. *Journal of Adhesive Dentistry* **2016**, *18*.
79. Gernhardt, C.; Kielbassa, A.; Hahn, P.; Schaller, H.G. Tensile bond strengths of four different dentin adhesives on irradiated and non-irradiated human dentin in vitro. *Journal of Oral Rehabilitation* **2001**, *28*, 814-820.

 doi:10.20944/preprints202309.0468.v1

80. Bernard, C.; Villat, C.; Abouelleil, H.; Gustin, M.P.; Grosgogeat, B. Tensile Bond Strengths of Two Adhesives on Irradiated and Nonirradiated Human Dentin. *Biomed Res Int* **2015**, *2015*, 798972, doi:10.1155/2015/798972.
81. Rodrigues, R.B.; Soares, C.J.; Junior, P.C.S.; Lara, V.C.; Arana-Chavez, V.E.; Novais, V.R. Influence of radiotherapy on the dentin properties and bond strength. *Clin Oral Investig* **2018**, *22*, 875-883, doi:10.1007/s00784-017-2165-4.
82. Munoz, M.A.; Garin-Correa, C.; Gonzalez-Arriagada, W.; Quintela Davila, X.; Häberle, P.; Bedran-Russo, A.; Luque-Martinez, I. The adverse effects of radiotherapy on the structure of dental hard tissues and longevity of dental restoration. *International journal of radiation biology* **2020**, *96*, 910-918.
83. Naves, L.Z.; Novais, V.R.; Armstrong, S.R.; Correr-Sobrinho, L.; Soares, C.J. Effect of gamma radiation on bonding to human enamel and dentin. *Supportive Care in Cancer* **2012**, *20*, 2873-2878, doi:10.1007/s00520-012-1414-y.