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CAROLINA SANDRA GARFIAS YAMASHITA

Desempenho *in-vitro* de um adesivo à base de MDP e livre de Bisfenol A na interação com a dentina sadia e a dentina afetada pela cárie

In-vitro performance of a Bisphenol A-free MDP-based adhesive and its interaction with sound and caries-affected dentin

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Orientador: Prof. Dr. Mario Fernando De Goes

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RESUMO

O estudo avaliou a resistência de união à microtração (µTBS) e a morfologia de união de dois adesivos à base de MDP — Scotchbond Universal e Scotchbond Universal Plus (livre de bisfenol A) — em dentina sadia e afetada pela cárie (DAC), considerando dois modos de aplicação e dois tempos de armazenamento na água.

Para o teste de microtração, as amostras foram distribuídas em oito grupos (n=9), de acordo com o tipo de dentina (sadia e DAC), os adesivos — Scotchbond Universal (SBU) e Scotchbond Universal Plus (SBUP) —, os dois modos de aplicação — condicionamento ácido (ER) e auto-condicionante (SE), e os dois tempos de armazenamento na água —24 horas e 1 ano. Os grupos ER foram condicionados com ácido fosfórico a 37% por 15 s. Todos os adesivos foram aplicados de acordo com as instruções do fabricante. Para o teste µTBS, a resina composta foi aplicada de forma incremental sobre a superfície tratada. Após 24 horas ou 1 ano, os palitos de 1 mm^2 foram testados a uma velocidade de 1 mm/min. Os dados foram analisados estatisticamente com o ANOVA três fatores, seguido do teste post-hoc de Bonferroni ($\alpha < 0.05$). Os modos de falha foram determinados usando a microscopia eletrônica de varredura (MEV). Para a análise da morfologia de união (n=3), os adesivos foram misturados com Rodamina-B e aplicados. Após 24 horas ou 1 ano, os espécimes foram imersos em fluoresceína e submetidos à microscopia confocal de varredura à laser (MCVL). O MEV também foi utilizado para análise da morfologia da interface (n = 3). A infiltração do adesivo e a infiltração da fluoresceína foram examinadas qualitativamente.

A DAC apresentou valores de µTBS significativamente menores (P<0,0001) aos da dentina sadia. O SBU no modo ER (DAC) apresentou os menores valores de µTBS para ambos períodos de armazenamento. O modo ER para ambos os adesivos mostrou fibras de colágeno expostas. O SBUP apresentou uma infiltração adesiva qualitativamente mais profunda na DAC, embora nenhuma diferença significativa no µTBS foi encontrada entre o SBUP (ER e SE) e o SBUSE após 1 ano de armazenamento na água. Não houve infiltração de fluoresceína após 24 horas para todos os grupos. Após 1 ano, SBU e SBUP utilizando o modo ER apresentaram sinais de degradação e infiltração de fluoresceína sob a camada híbrida (CH).

O adesivo Scotchbond Universal Plus apresentou melhor difusão na dentina afetada pela cárie e maior resistência de união em ambos os modos de aplicação às 24 horas. O modo autocondicionante (SE) é recomendado para ser usado com os adesivos Scotchbond Universal e Scotchbond Universal Plus em dentina sadia ou afetada pela cárie.

Palavras-chave: cárie dentária, dentina, adesivos dentários, adesão, resistência à tração, materiais dentários, microscopia eletrônica de varredura

ABSTRACT

The aim of the study was to evaluate the microtensile bond strength (μTBS) and adhesive interface micromorphology of two MDP-based adhesives — Scotchbond Universal and Scotchbond Universal Plus (bisphenol A-free) — on sound and caries-affected dentin (CAD), considering two application modes and different water storage periods.

For the microtensile test, the samples were distributed into eight groups (n=9), according to the type of dentin (sound and CAD), adhesives — Scotchbond Universal (SBU) or Scotchbond Universal Plus (SBUP) —, the two application modes — etch-and-rinse (ER) or self-etching (SE), and the two aging times — 24 h or 1 year. The ER groups were etched with 37% phosphoric acid for 15 s. All adhesives were applied according to the manufacturer's instructions. For the μTBS test, the composite resin was applied incrementally over the treated surface. After 24 hours or 1 year, the 1 mm^2 sticks were tested at a speed of 1 mm/min. Data were statistically analyzed using three-way ANOVA followed by the Bonferroni post-hoc test ($\alpha < 0.05$). Failure modes were determined using scanning electron microscopy (SEM). For analysis of bond morphology (n=3), adhesives were mixed with Rhodamine-B and applied. After 24 hours and 1 year, the specimens were immersed in fluorescein and subjected to confocal laser scanning microscopy (CLSM). SEM was also used to analyze interface morphology (n=3). Adhesive diffusion and fluorescein infiltration were examined qualitatively.

CAD showed μTBS mean values significantly lower ($P<0.0001$) than those of the sound dentin for all groups at 24 h. The self-etching mode on sound dentin showed no signs of degradation or fluorescein infiltration. SBU in the ER mode (CAD) showed the lowest μTBS mean values in both storage periods. The ER mode for both adhesives showed exposed collagen fibrils and fluorescein infiltration underneath the hybrid layer of the sound and CAD overtime. Although SBUP showed a deeper adhesive diffusion into the CAD, no significant difference in μTBS was found between SBUP (ER and SE) and SBUSE after a year of water storage.

The self-etching mode is recommended to be used for the Scotchbond Universal and Scotchbond Universal Plus adhesives in either sound or caries affected dentin. Scotchbond Universal Plus showed better adhesive diffusion into the caries-affected dentin and greater bond strength with both application modes at 24 h.

Keywords. caries-affected dentin, dentin, longevity, adhesion, microtensile bond strength, hybrid layer, universal adhesives, confocal laser scanning microscopy, scanning electron microscopy

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1. INTRODUCÃO

Os avanços na tecnologia dos adesivos dentários revolucionaram a prática clínica, permitindo procedimentos de mínima intervenção (Degrange M, Roulet JF, 1997). Os métodos tradicionais que exigiam a remoção da dentina sadia para obter retenção mecânica foram substituídos por procedimentos que permitem a adesão química e micromecânica à dentina afetada pela cárie (DAC) (Degrange M, Roulet JF, 1997).

A DAC é aquela localizada imediatamente sob a dentina infectada pela cárie. Embora seja ligeiramente desmineralizada, ainda é passível de remineralização (Nakajima M, et al., 2011). A DAC apresenta características que reduzem ou interferem na adesão, como maior teor de água (Spencer P, et al., 2005), perda de minerais (Ogawa K, et al., 1983, Angker L, et al., 2004), alterações na matriz de colágeno (Nakajima M, et al., 2011) e obstrução tubular por aposição de minerais como resposta à agressão bacteriana (Nakajima M, et al., 2011). Essas alterações podem reduzir a qualidade da difusão do adesivo, (Pashley DH, et al., 2004, Erhardt MC, et al., 2008) gerar infiltrações nas interfaces dentina-adesivo, e reduzir as propriedades mecânicas como descrito anteriormente por outros estudos (Erhardt MC, et al., 2008, Gutiérrez MF, et al., 2022, Marchesi G, et al, 2014).

Uma tendência atual é a usar adesivos de frasco único, como os adesivos universais multimodo (UAs), que se tornaram muito populares na odontologia restauradora devido às suas características químicas e sua versatilidade de aplicação (Perdigão J., 2020, Rosa WL, Piva E, Silva AF, 2015, Chen C, et al., 2015). Vários adesivos universais contêm o 10-metacriloiloxidecil dihidrogenofosfato (10-MDP), um monômero funcional que interage com a hidroxiapatita (Yoshida Y, et al., 2004) para formar sais de cálcio hidroliticamente estáveis, aumentando a longevidade das restaurações dentárias (Yoshida Y, et al., 2004, Yoshida Y, et al., 2012, Yoshihara K, et al., 2013). Sua composição química facilita ao dentista a decisão sobre o modo de aplicação (Chen C, et al., 2015, Hanabusa M, et al., 2012). No caso do modo 'etch-and-rinse' (ER), o ácido fosfórico é conhecido por ser muito agressivo em estruturas como a dentina afetada pela cárie (Nakajima M, et al., 2011), causando uma desmineralização dentinária mais profunda do que qualquer adesivo pode infiltrar (Pashley DH, et al, 2011). A infiltração incompleta do adesivo na matriz de colágeno gera uma suscetibilidade a degradação enzimática e hidrolítica desta zona (Pashley DH, et al, 2011, Nakajima M, et al., 2005, Yoshiyama M, et al., 2002). Controversamente, foi relatada uma maior resistência de união para estratégias ER na dentina afetada pela cárie; no entanto, apenas estudos de curto prazo mostraram esses resultados e nenhum investigou adesivos universais em dentes humanos com cáries que se desenvolveram naturalmente na boca. (Yoshiyama M, et al., 2002, Ceballos L, et al., 2003, Isolan CP, et al., 2018, Yazici AR, et al., 2004, Yokota H, 2006).

Por outro lado, o modo de aplicação autoacondicionante ou 'self-etching' possui a capacidade de desmineralizar e infiltrar a dentina simultaneamente na mesma profundidade, permitindo uma maior difusão do adesivo na matriz de colágeno (Van Meerbeek B, et al., 2011). No entanto, na dentina afetada pela cárie, o adesivo autocondicionante ultra-leve (pH >2.5) reduz sua capacidade de dissolver e remover os depósitos minerais nos túbulos dentinários, condições que resultam na ausência de formação de tags de resina e infiltração incompleta. Desta forma, formam-se regiões de pobre hibridização que impactam negativamente na resistência adesiva e na longevidade da interface adesiva (Yoshiyama M, et al., 2002).

A maioria dos adesivos dentários contêm o Bisfenol A (BPA) que é o componente central do Bis-GMA (BPA glicidil metacrilato) (Lovell LG, et al., 1999). O Bis-GMA é o monômero de mais utilizado nos adesivos dentários devido à suas altas propriedades mecânicas (Lovell LG, et al., 1999). O BPA foi detectado na saliva e na urina de pacientes após procedimentos odontológicos. Apesar do seu baixo nível, a presença de BPA é preocupante porque pode desencadear efeitos estrogênicos (Becher R, et al., 2018, Kingman A, et al., 2012, De Nys S, et al., 2021). Em 2020, foi introduzido no mercado odontológico um adesivo livre de bisfenol A baseado em 10-MDP e um novo monômero (ácido 2-propenóico, 2 metil-, diésteres com 4,6-dibromo-1,3-benzenodiol 2-(2-hedroxietoxi) etil 3-hidroxipropil diéteres) substituiu o Bis-GMA (3M Scotchbond universal plus adhesive/Technical product profile, 2020). De acordo com o fabricante, o adesivo apresenta menor viscosidade, quando comparado com seu antecessor, proporcionando melhor infiltração nos tecidos dentais e maior resistência de união à dentina sadia e afetada pela cárie (3M Scotchbond universal plus adhesive/Technical product profile, 2020, Thalacker C, et al., 2020).

Nenhum estudo foi encontrado para investigar o desempenho mecânico e a capacidade de infiltração deste adesivo livre de bisfenol A na interface adesiva da dentina afetada pela cárie. Sendo assim, o objetivo do presente estudo foi avaliar a resistência de união e as características morfológicas do adesivo isento de bisfenol A comparado com seu antecessor em dentina sadia e afetada pela cárie, considerando dois modos de aplicação e dois períodos de armazenamento na água. As hipóteses de pesquisa deste estudo foram (i) o tipo de dentina, (ii) o tipo de adesivo e o modo de aplicação, (iii) o tempo de armazenamento na água não afetariam a resistência de união e as características morfológicas da interface adesivo/dentina.

2. ARTIGO: In-vitro performance of a Bisphenol A-free MDP-based adhesive and its interaction with sound and caries-affected dentin

Running title: Bisphenol A-free universal adhesive improved bonding diffusion on caries-affected dentin

Artigo submetido ao periódico Operative Dentistry Journal (Anexo 2)

Autores: Garfias CS, De Goes MF.

Abstract

Purpose: To evaluate the bond strength and adhesive interface micromorphology of a bisphenol A-free universal adhesive in sound and caries-affected dentin, considering the etch-and-rinse and self-etching application modes and different water storage periods.

Materials and Methods: Human molars with sound and caries-affected dentin (CAD) had their surfaces prepared and bonded with Scotchbond Universal (SBU) or Scotchbond Universal Plus (SBU**P**) using the etch-and-rinse (ER) or self-etching (SE) modes. Resin-dentin bonded specimens (1 mm^2) were stored in water for 24 hours and 1 year and tested for microtensile bond strength (μTBS). The failure images were analyzed through scanning electron microscopy (SEM). Data were submitted to the three-way ANOVA and post-hoc Bonferroni test ($\alpha < 0.05$). Three additional teeth were prepared for each group for evaluation of the adhesive diffusion into dentine by dyeing the adhesives with a fluorochrome (Rhodamine B). After longitudinal sectioning, the generated interfaces were stored for 24 h and 1 year, immersed in fluorescein dye and examined under confocal laser scanning microscopy (CLSM). Adhesive interface morphology was also analyzed using SEM (n=3).

Results: CAD showed μTBS mean values significantly lower ($P<0.0001$) than those of the sound dentin for all groups at 24 h. The self-etching mode on sound dentin showed no signs of degradation or fluorescein infiltration. SBU in the ER mode (CAD) showed the lowest μTBS mean values in both storage periods. The ER mode for both adhesives showed exposed collagen fibrils and fluorescein infiltration underneath the hybrid layer of the sound and CAD overtime. Although SBU**P** showed a deeper adhesive diffusion into the CAD, no significant difference in μTBS was found between SBU**P** (ER and SE) and SBUSE after a year of water storage.

Conclusion: The self-etching mode should be used for the Scotchbond Universal and Scotchbond Universal Plus adhesives in either sound or caries affected dentin. Scotchbond Universal Plus showed better adhesive diffusion into the caries-affected dentin and greater bond strength with both application modes at 24 h.

Clinical significance: The clinical use of Scotchbond Universal Plus adhesive (bisphenol A-free) can be encouraged to be used using the self-etching approach on sound and caries-affected dentin.

Keywords. caries-affected dentin, dentin, longevity, adhesion, microtensile bond strength, hybrid layer, universal adhesives, confocal laser scanning microscopy, scanning electron microscopy

1. Introduction

The long-term adhesive bonding at the resin-tooth interface determines the clinical success of a resin composite restoration.[1] The mechanical bonding of resin composites to acid-etched enamel has been well-established in the literature;[1] however, their bonding to dentin remains questionable, regardless of the adhesive application modes — etch-and-rinse and self-etching.[2–13]

Following the removal of the infected dentin, the cavity floor typically consists of caries-affected dentin (CAD).[3] This substrate demonstrates lower bonding efficacy compared to sound dentin due to morphological, chemical, and physical alterations. CAD is softer than sound dentin as a result of the partial demineralization within the intertubular matrix despite the intratubular deposition of calcium phosphate crystals.[2,3] In addition, CAD is characterized by modifications in the collagen matrix and higher water content compared to sound dentin.[2,3]

Universal adhesives (UAs) have become popular in restorative dentistry due to their chemical properties and application versatility.[2, 14, 20] UAs usually contain 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP), a functional monomer that interacts with hydroxyapatite [15] to form hydrolytically stable calcium salts, increasing the longevity of dental restorations.[16–18] Their chemical composition makes it easier for dental practitioners to decide on the application mode.[14, 19, 20, 21] In the case of the etch-and-rinse mode, the phosphoric acid is known to be aggressive in dentin [3] causing a dentinal decalcification deeper than any adhesive can infiltrate,[22] leading to an incomplete adhesive infiltration into the exposed collagen matrix.[13,22,23] Controversially, higher bond strengths have been reported for etch-and-rinse strategies in sound and CAD; however, only short-term studies showed those results [8, 9, 24–26] and none of them investigated UAs on human teeth with dental caries that developed naturally within the mouth.

In the self-etching mode, dentinal demineralization and adhesive infiltration occur simultaneously. Because demineralization in sound dentin is less intense in this mode, a complete adhesive infiltration is expected.[27] Unlike the etch-and-rinse mode, the self-etching mode reduces their ability to dissolve and remove the mineral deposits in the dentinal tubules, conditions that result in no resin tag formation and incomplete adhesive diffusion in CAD [8]. Thus, leaving regions of poor hybridization that impact negatively on bond strength and fails to halt the accelerated degradation of the adhesive interface over time.[8, 9, 13]

Bisphenol A (BPA) is the core component of Bis-GMA (BPA glycidyl methacrylate), which is the most widely used monomer in dental adhesives due to its high mechanical strength and compatibility with other resin-based materials.[28] BPA, though detected at low levels in patients' saliva and urine after dental procedures, is concerning due to its potential estrogenic effects.[29–31] A bisphenol A-free adhesive based on 10-MDP along with a new monomer (2-propenoic acid, 2 methyl-, diesters with 4,6-dibromo-1,3-benzenediol 2-(2-hedroxyethoxy) ethyl 3-hydroxypropyl diethers) has been developed as a Bis-GMA replacement.[32] According to its manufacturer, it provides great dentinal monomer diffusion, low viscosity and high bond strength to both sound and caries-affected dentin.[32,33]

No study has been found to investigate the mechanical performance and the adhesive diffusion of this bisphenol A-free UA in the bonding interface of caries-affected dentin. Therefore, the aim of the present study was to evaluate the bond strength and the morphological characteristics of a bisphenol A-free UA in sound and caries-affected dentin, considering the etch-and-rinse and self-etching application modes and different water storage periods. The null hypotheses were as follow: (i) the type of dentin, (ii) the type of adhesive and application mode, and (iii) aging would not affect the bond strength and morphological characteristics of the adhesive/dentin interface.

2. Materials and Methods

Freshly extracted human molars — 120 sound and 120 caries-affected dentin (CAD)— were collected under a protocol approved by the local ethics committee (#5.048.095). A power calculation was carried out to determine the minimal number of teeth (n=9) required for the microtensile bond strength test ($P<0.05$; power value: 0.80; and effect size: 0.5). The hard and soft tissue deposits were removed using a scalper and cleaned using a rubber cup with pumice solution and then stored in 0.5% chloramine-T at 4 ºC up to testing.

The sound teeth had their coronal dentin sectioned 1.5 mm below the occlusal fossa using a diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) under water cooling (Fig. 1). Their mid-coronal dentin surface was exposed and sandpapered (wet 600-grit SiC) for 60 s resulting in a standardized smear layer and roughness. The surrounding enamel was removed using a diamond bur (high-speed handpiece).

The caries-affected teeth had their occlusal caries lesions exposed by removing the occlusal enamel and superficial dentin using a low-speed diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) under water cooling. The dentin was stained (Caries Detector; Kuraray Medical Inc., Tokyo, Japan) for visual detection. The dark pink-to-red dentin was classified as caries-infected and the light pink dentin as CAD, the latter of which ranged from mid to deep dentin (±1.5 mm from the pulp). The caries-infected dentin was then removed (600-grit SiC sandpaper) under running water resulting in CAD with a slightly hard flat surface and brownish-to-yellowish color.[34–36] To isolate the CAD, the surrounding sound dentin was removed using a diamond bur (Fig. 1). The coronal pulp of each tooth was fully removed using hand instruments and the pulp chamber was cleaned with tap water and etched using 37% phosphoric acid for 15 s. The pulp chamber was then sealed using a bonding agent and filled with flowable and resin composite.

2.1. Bonding Procedures

The previously prepared teeth were sectioned into two halves (Fig. 1) using a diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) to follow a split tooth design. The halves (Table 1) were assigned to the experimental groups (n=9), according to the type of dentin – sound or caries-affected dentin, to the application protocol – one half for Scotchbond Universal Plus/SBU**P** and the other half for Scotchbond Universal/SBU (3M ESPE - Deutschland Gmbh, Seefeld, Germany) and to the etch-and-rinse mode (ER) or self-etching mode (SE), and to the water storage time – 24 h or 1 y.

Table 2 shows the chemical composition, manufacturers, and application modes of the universal adhesive systems. A 6-mm-thick block of resin composite (Filtek Supreme Ultra 3M ESPE; St. Paul, MN, USA) was incrementally built up onto each treated dentin surface. Every 2-mm-thick increment was light cured for 20 s (Deep Cure, 3M ESPE; St Paul, MN, USA) at 1200 mW/cm^2. The resin blocks bonded to the dentin were then stored in distilled water at 37 °C for 24 h.

2.2. Microtensile Bond Test

Each resin composite block (sound and CAD) was longitudinally sectioned using a water-cooled diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) to produce 6 to 8 beams (1.0 ± 0.1 mm^2 cross-sectional area) for the sound, and 3 to 4 beams for CAD. The specimens (beams) were randomly subdivided and stored in distilled water at 37 °C for 24 h and 1 y. For the 1-year stored specimens, the solution was changed weekly.[37] After each storage period, the specimens were individually fixed to a jig with cyanoacrylate glue and tested for microtensile bond strength (µTBS) using a tensile testing machine (EZ-test; Shimadzu Co., Kyoto, Japan) at a crosshead speed of 1.0 mm/min until failure (Fig. 1). After failure, a digital caliper was used to measure each specimen's cross-sectional area of the failure and calculate the μTBS values. A μTBS mean value was obtained for each dentin condition (sound and CAD) and expressed in Mega Pascal (MPa).

Normality and homoscedasticity were confirmed by Shapiro-Wilks and Levene, respectively. Data were statistically analyzed (IBM SPSS Statistics software, Armonk, NY, USA) using three-way ANOVA (dentin type vs. adhesive system vs. storage time) followed by the post-hoc Bonferroni test ($\alpha = 0.05$). When the specimen broke precociously, it was recorded as a pretesting failure (PTF), excluded from the statistical analysis, and described in the results (Table 3).

2.3. Scanning electron microscopic (SEM) analysis of failure modes

All fractured surfaces were sputter coated with gold/palladium and submitted to scanning electron microscopy (SEM; JEOL-5600 LV, Tokyo, Japan) at 15 kV and magnifications of 85x and 3000x. The failure modes were classified as follows: type I — cohesive failure in hybrid layer; type II — cohesive failure in resin composite; type III — cohesive failure in dentin; and type IV — mixed cohesive failure (adhesive, hybrid layer, resin composite, and dentin).

2.4. Bonding Procedure and confocal laser scanning microscopy CLSM Analysis

For the resin-dentin bonding interface analysis with CLSM (n=3), the adhesives were doped with Rhodamine B (0.1wt%, Sigma Chemicals; St Louis, MO, USA) according to a previously published protocol[37-39]. Both adhesives were applied to the dentin (sound and caries-affected) surface according to the manufacturer's instructions (Table 2) and a 1-mm-thick layer of flowable composite (A2 Filtek Z350 XT flowable resin, 3M ESPE; St Paul, MN, USA) was built up onto the dentin and light cured for 20 s. After 24 hours of water storage at 37ºC, the dentin-resin interface was transversely sliced into 1-mm-thick slabs using a water-cooled diamond saw. Each slab was polished with 600, 800, 1200, and 2000-grit SiC abrasive sandpaper (Carborundum Abrasives, Recife, PE, Brazil) under running water. Between each polishing step, the specimens were ultrasonically cleaned (Unique Ind. Co. and Electronic Products Ltda, Sao Paulo, SP, Brazil) for 3 minutes. Next, 0.3-µm, and 0.05-µm diamond paste (UK Buehler LTD, Lake Bluff, IL 60044, USA) on polishing felts was used to complete the polishing procedure. Finally, the specimens were polished with a felt without diamond paste for 5 minutes under water cooling to ensure the removal of surface debris.

After 24h and 1 year, the specimens were immersed in 1 wt.% aqueous/ethanol fluorescein for 3 hours and then analyzed using confocal laser scanning microscopy (CLSM; TCS SP5AOBS, Leica Microsystems, Wetzlar, Germany), with an oil-immersion lens (63X/1.4 NA) and argon (488 nm) and helium (543 nm) laser illumination. CLSM analysis included 10-µm z-step optical sections at a depth of 1 µm.

2.5. Bonding Procedure and Analysis for SEM

After adhesive application and light curing, the teeth (n=3) were restored with flowable resin composite (Filtek Supreme- 3M ESPE, St. Paul, MN, USA) and stored in water for 24 h. After water storage, they were sectioned into two 2-mm-thick slabs to expose the bonded surfaces and stored in water for 24 h or 1 year (Fig. 1). The slabs were then embedded in epoxy resin (Epoxy cure, Buehler Inc.) and polished with 1200- and 2000-grit silicon-carbide (SiC) abrasive paper (Norton AS; São Paulo, Brazil) and a polishing cloth with 1-µm diamond paste (Buehler Ltd, Lake Bluff, IL, USA). A water-moist cloth was used for the final polishing.

The specimens (slabs) were then ultrasonically cleaned for 3 min, demineralized in 50% phosphoric acid for 25 s, deproteinized in 0.1% NaOCl solution for 20 min, dehydrated in ascending ethanol (25%, 50%, 75%, and 100%; 30 min for each concentration), dried with

HMDS for 10 min, and stored in dried silica. After chemical dehydration, the bonding interfaces were sputter coated with gold/palladium (SCD 050; Balzers) at 40 mA for 120 s and submitted to SEM analysis (JSM 5600LV; JEOL, Tokyo, Japan) at 15 kV.

3. Results

3.1. Microtensile bond strength (μTBS)

Table 3 shows μTBS mean values, standard deviations (MPa), and number of specimens and pre-test failures (PTF) for all groups and storage periods (24 h and 1 y). A significant ($P<0.05$) interaction was observed between the substrate and aging periods. The μTBS values obtained for the caries-affected dentin (24 h storage) were significantly lower ($P<0.0001$) than those of the sound dentin, regardless of the adhesive system and application mode. Unlike the sound dentin (24 h storage), CAD showed a significant reduction in μTBS for SBUER (40%), SBUSE (36%), and SBU**P** (25%) in both application modes (Table 4).

A significant reduction in μTBS was observed for both sound and CAD at 1-year water storage (Tables 3 and 4). Only SBUSE-C showed no alteration in bond stability after 1 year of storage. No significant difference was observed between SBUSE-C and the novel adhesive considering both application modes (1-year storage). SBUER-C showed the lowest μTBS values in both storage periods. No significant difference was observed between SBU**P**SE and SBUSE (1-year storage), considering the sound and caries-affected dentin (Table 3).

3.2. Failure modes— SEM analysis

Figures 2 (24-hour) and 3 (1-year storage) illustrate a graphic distribution (%) of the failure modes concerning all groups. At 24 h, SBU**P** in both application modes showed prevalence of the cohesive failure in the hybrid layer (Type I). SEM analysis showed the dentinal tubules filled with SBU**P** (etch-and-rinse), with some collagen fibers spaces partially unfilled (Fig. 4).

SBU**P**ER-C (both aging periods) showed prevalence of the mixed failure (Type IV — cohesive fracture in the adhesive, hybrid layer, dentin, and resin composite), where the fracture occurred at the bottom of the hybrid layer, with the collagen fibers partially exposed and the resin tags fractured at different sites (Figures 5 and 7).

The mixed failure (Type IV) was the most prevalent in SBUSE, considering both dentinal conditions and storage periods. SBUSE-C (Figs. 5-D1 and 7-D1) showed mixed failures at the bottom of the hybrid layer with visibly empty dentinal tubules and the intratubular area partially uncovered. SBUER — in both dentinal conditions and storage periods — showed cohesive fractures in the hybrid layer (Type I), with non-encapsulated collagen fibers and some empty tubules, especially in CAD (Figs. 4, 5, 6, and 7).

3.3. Bonding morphology with CLSM and SEM

Hybrid layer (HL) formation was observed for both adhesives, either in the etch-and-rinse (ER) or in the self-etching (SE) application modes (Figs. 8–12). In the ER mode, both adhesives showed thicker hybrid layers and longer well-defined resin tags in CAD (Figs. 8–12). SBU**P** using the SE mode showed similar infiltration morphology to the ER mode (Fig. 9). In both dentinal conditions, SBU**P**SE showed resin tags longer than those observed in SBUSE (Fig. 9 and 11). Adhesive infiltration (red dye — Figs. 8 and 9) resulting in full sealing of the bonding interface, was observed for all the groups at 24 h for both application modes. SBU**P** infiltration for both application modes was observed in the intra- and inter-tubular dentin of the partially demineralized and mineralized CAD, showing long resin tags and thick HL (Figs.9, 11 and 12). The ER mode for both adhesives showed signs of degradation and fluorescein infiltration underneath the HL after 1-year (Fig. 11).

4. Discussion

In the real clinical scenario, the dental clinician often encounters caries-affected dentin, which is partially demineralized and requires no removal. In the present study, the Bis-GMA-based (SBU) and the BPA-free (SBU**P**) adhesives showed significantly higher μTBS mean values and better morphological characteristics for the sound dentin. Caries-affected dentin showed the lowest μTBS mean values and displayed interfacial bonding defects when using the etch-and-rinse mode with SBU. Based on these findings, the first and second null hypotheses were rejected.

The etch-and-rinse mode has been reported to demineralize the dentin and expose the collagen fibrils to a greater extent than the self-etching,[6, 34, 36] a condition that might lead to incomplete adhesive infiltration.[36] These findings were in accord with those observed in the present study, in which the caries-affected dentin in the ER mode showed empty dentinal tubules and exposed collagen matrix (SEM), especially for SBU (Figs. 5C–C1 and 7C–C1). In addition, SBUER in the sound and caries-affected dentin showed signs of low-quality hybridization, characterized as a yellowish profile beneath the hybrid layer, indicating sites of interfacial defects and incomplete sealing (Fig. 11).

These outcomes might be associated with the largest number of pre-test failures, the greatest infiltration of fluorescein (1 y) and the lowest μTBS mean values observed for SBUER in the caries-affected dentin (Table 3). The alterations in the chemical and morphological characteristics of the caries-affected dentin after etching might account for these low bond strength mean values. CAD, partially demineralized, showed an irregular and thicker hybrid layer enriched with organic components (Figs. 9 and 12); this finding is accord with those previously reported. [3, 6, 7, 8, 13, 23, 36]

SBU**P**ER showed a deep monomer diffusion into the caries-affected dentin with thicker hybrid layers and longer and more numerous resin tags than those observed for SBUER (Fig.

9). Such characteristic is represented by a slightly reflective signal (CLSM) from the bottom of the hybrid layer to the adhesive layer. The red resin tags indicate that the adhesive monomer infiltrated the dentinal tubules and their branches and reached the caries-affected dentin. The funnel-shaped resin tags underneath the adhesive layer characterize a sealed resin-dentin bonding interface while the high reflective green signal identifies the caries-affected dentin area (Figs. 9 and 11).

The μTBS (24 h) was reduced in the caries-affected dentin regardless of the application mode (Table 3). Although the adhesive (SBU**P**) and their tags occupied a large area of the mineral structure of the dentin lost after the caries process, it was insufficient to provide a mechanical behavior (μTBS) similar to that of the sound dentin, whose structure is fully mineralized. Despite that, concerning the sound and caries-affected dentin, SBU**P** in CAD showed a reduction by 25% in μTBS when compared to the sound dentin (24 h), while SBU showed a reduction by approximately 40% (Table 4) in both application modes, suggesting that SBU**P** had a mechanical behavior (μTBS) considerably better than that of SBU (Table 4). The 40% reduction in μTBS has also been reported in previous studies using different adhesives at 24 h in the caries-affected dentin.[6, 7, 9, 11, 13, 15–20, 22, 36, 43–49]

During the carious process, minerals are deposited in the dentinal tubules, a condition that might affect the infiltration of the resin monomers.[13, 50, 51, 52] In the present study, despite the mineral obstruction, SBU**P** effectively infiltrated the tubules of the caries-affected dentin, regardless of the application mode (Fig. 9). The novel monomer in the chemical composition of SBU**P** might account for its greater infiltration into the demineralized areas and dentinal tubules in the SE and ER modes (Fig. 9). According to its manufacturer, SBU**P**'s viscosity was adjusted using non-setting silica fillers and solvents similar to those in SBU.[53] SBU**P** has been reported to be less viscous than SBU,[53] a condition that might have facilitated its infiltration into caries-affected dentin, as indicated by the numerous lateral branches visible in the SEM images (Figs. 5 and 12). This deeper infiltration of SBU**P** might have reinforced the caries-affected dentinal structures in the first 24 h, increasing the μTBS in both application modes (Table 3).

Despite the alterations in the chemical and morphological characteristics of the caries-affected dentin, the SBU**P**SE-C showed μTBS mean values significantly higher than those obtained for SBUER-C. This finding might account for the prevalence of the fracture mode Type I (cohesive in hybrid layer) obtained for SBU**P**SE in the caries-affected dentin (Fig. 2). SBU**P**ER-C showed prevalence (Fig. 2) of the mixed failure mode type IV (cohesive fracture in adhesive, hybrid layer, dentin, and resin composite). The SEM images of the fracture at the bottom of the hybrid layer showed that the collagen fibrils were not filled with adhesive and that the resin tags fractured in different sites (Figs. 5 and 7). In this case, the higher prevalence of the failure mode type IV might be due to the morphological characteristic of CAD, which is more porous than the sound dentin. This condition facilitates the diffusion of the inorganic conditioners, such as the phosphoric acid (etch-and-rinse), which causes deeper demineralization.

The bonding interface is composed of three dentinal conditions — slightly filled with adhesive, fully exposed, and partially demineralized. In a deeper demineralized zone, the resin monomer scarcely infiltrates the bottom of the exposed collagen matrix of the dentin.[3] These findings were morphologically visible (Figs. 5A1, 7C1, 7D1) in the present study, showing the collagen fiber mesh fully unfilled with adhesive. However, this morphological characteristic had no impact on the μTBS. The lower μTBS values (Table 3) of the caries-affected dentin, compared with the sound dentin, might be due to a reduction in the cohesive strength of the dentin. This reduction might be associated with the alteration in the chemical and morphological characteristics of the caries-affected dentin.

The 1-year water storage significantly reduced the μTBS of the adhesives in both dentinal substrates and application modes, except for SBUSE-C, and affected the morphology of the bonding interface when using the ER mode (Figs. 10 and 12) therefore, the third null hypothesis was rejected. SBU in the caries-affected dentin at 24 h showed a significant reduction in μTBS (Table 3) in the ER and SE modes and loss of interface integrity in the ER mode (Fig. 10). At 1-year water storage, no statistical difference in μTBS was observed between the sound and caries-affect dentin in the SE mode (Table 3). The CLSM images (Fig. 11) showed a full sealing of the dentin, with the resin monomers fully infiltrated in the demineralized area of the caries-affected dentin; a spectral overlap of both dyes (red and green) resulted in a yellowish profile, indicating partial dentin demineralization (CAD).

This novel monomer (2-propenoic acid, 2 methyl-diesters with 4,6-dibromo-1,3-benzenediol 2-(2-hedroxyethoxy) ethyl 3-hydroxypropyl diethers) in SBU**P** is of hydrophilic and hydrophobic nature.[32] This amphiphilic characteristic, the weight of the molecule and the hydroxyl groups in its chemical composition might increase its hydrophilicity and wettability, conditions that might account for its deeper infiltration in the caries-affected dentin (Fig. 4). The 1-year storage of the specimens in distilled water, which was changed weekly, might have increased the water sorption by the hydrophilic groups of the molecule, a condition that might have reduced the bond strength and increased the bonding interface degradation of both adhesives in both application modes (Table 3). The hydrophilic nature of the SBU**P** adhesive, in contrast to the higher hydrophobicity of Bis-GMA in SBU combined with other hydrophilic components in the same bottle, may have resulted in a more hydrophobic surface layer in SBU, potentially explaining the stability it demonstrated over the year using the SE mode in the CAD.

Rhodamine B (red dye) and fluorescein (green dye) were used to make the sealing visible in the dentin bonding interface (Figs. 8–11). The yellowish profile (CLSM images), resulting from an interaction of the green dye with the red dye on the adhesive surface, indicates interfacial defects or partial dentin demineralization, which are common characteristics of the caries-affected dentin, with greater permeability when compared with that of the sound dentin. In these dentin demineralization sites (CAD), the fluorescein (green dye) was absorbed in different intensities (Figs. 9 and 11). The deeper the cavity and the greater the loss of minerals, the more intense was the fluorescein absorption. The degree of demineralization varied among the teeth (CAD) and generated different reflective signal intensities (fluorescein: green dye). This might be due to the naturally-occurring caries process adopted in the present study. Figure 9 shows different intensities of the reflective green signal for SBU**P**ER-C (low), SBUSE-C (medium), and SBUER-C (high). It also shows a spectral overlap of both dyes (red and green) resulting in a yellowish profile (SBU**P**SE-C). This green reflective signal, regardless of its intensity, indicates that the caries-affected dentin was partially demineralized.

All images (Figs. 9 and 11) obtained for the caries-affected dentin displayed a uniform dark line in the hybrid layer (blue arrow). This dark line might be misinterpreted as a gap formation in the bonding interface. Instead of a gap or an adhesive failure in the hybrid layer, it was identified as a technical artifact, or rupture, resulting from the specimens' preparation, particularly when the dentin-resin interface section was transversely sliced into 1-mm-thick slabs or when the slabs were polished (grit SiC sandpaper). This dark line is also visible in the resin tags (Figs. 9 and 11). Some resin tags in SBU**P**ER-C are visibly connected to the adhesive layer while others seem to start in the hybrid layer (Figs. 9 and 11).

The caries-affected dentin showed μTBS mean values significantly lower than those of the sound dentin, concerning both adhesives and when using the etch-and-rinse mode. In both application modes, SBU**P** infiltrated more deeply and fully sealed the inter- and intra-tubular dentinal areas in the sound and caries-affected dentin (Figs. 4 and 5). The 1-year water storage (Table 3 and Figs. 11 and 12) significantly reduced the μTBS of both adhesives and affected the morphology of the bonding interface in both dentinal substrates. The self-etching mode for both adhesives and substrates would be the ideal mode for clinical application, as it demonstrated superior long-term performance.

5. Conclusions

Within the limitations of this current study, it was concluded that:

1. Scotchbond Universal Plus adhesive (BPA-free) infiltrated deeper into the inter- and intra-tubular areas on the caries-affected dentin; however, it did not reflect in higher long-term bond strength.
2. The use of acid etching affected negatively on the bond strength and interface integrity of the caries-affected dentin.
3. Aging significantly reduced the bond strength of both adhesives on both types of dentin, except when using the Scotchbond Universal adhesive with the self-etching mode.

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Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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FIGURES

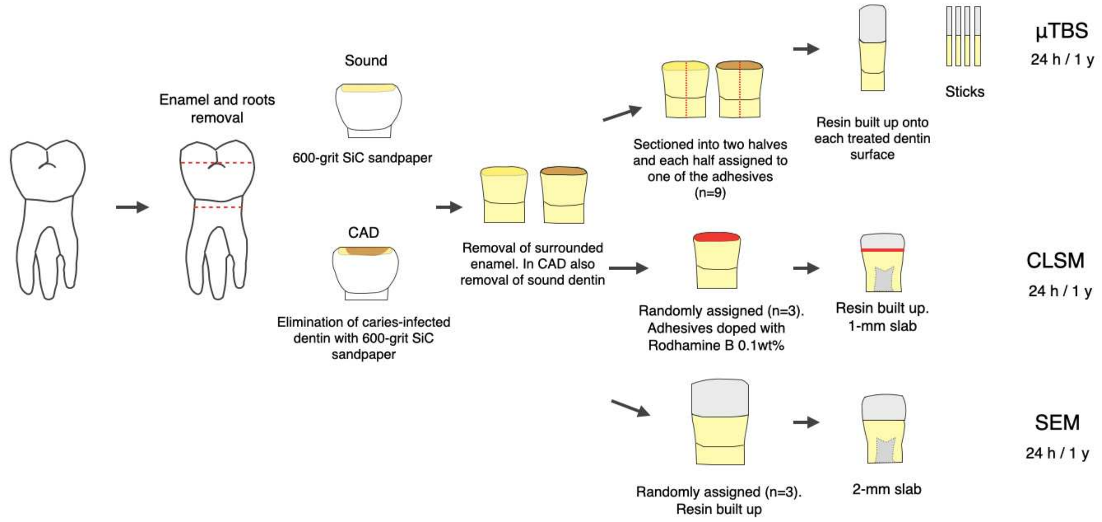

Figure 1. Schematic illustration showing the specimen preparation for microtensile bond strength test (µTBS) and interface morphology analysis by CLSM and SEM.

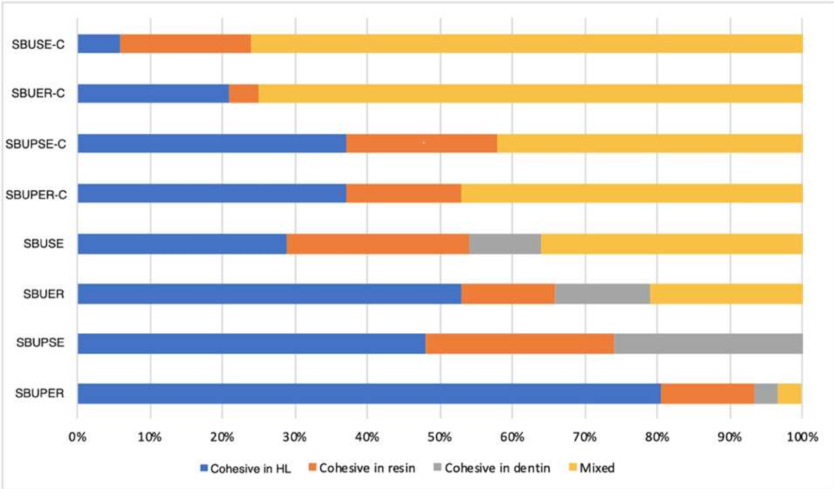

Figure 2. Failure mode distribution (%) of the experimental groups on sound dentin and caries-affected dentin at 24 h storage. Type I: cohesive failure in the hybrid layer; Type II: cohesive failure in resin composite; Type III: cohesive failure in dentin; Type IV: mixed failure (cohesive in the adhesive, hybrid layer, dentin, and resin composite).

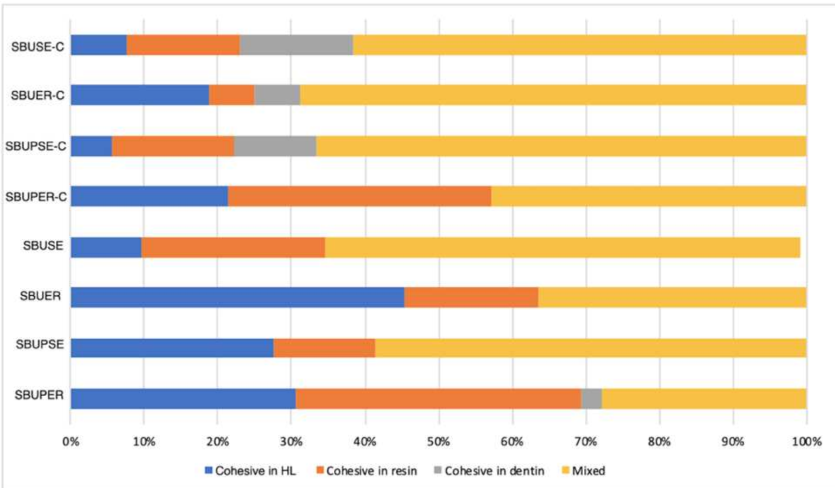

Figure 3. Failure modes distribution (%) of the experimental groups on sound and caries-affected dentin at 1-year water storage. Type I: cohesive failure in the hybrid layer; Type II: cohesive failure in resin composite; Type III: cohesive failure in dentin; Type IV: mixed failure (cohesive in the adhesive, hybrid layer, dentin, and resin composite).

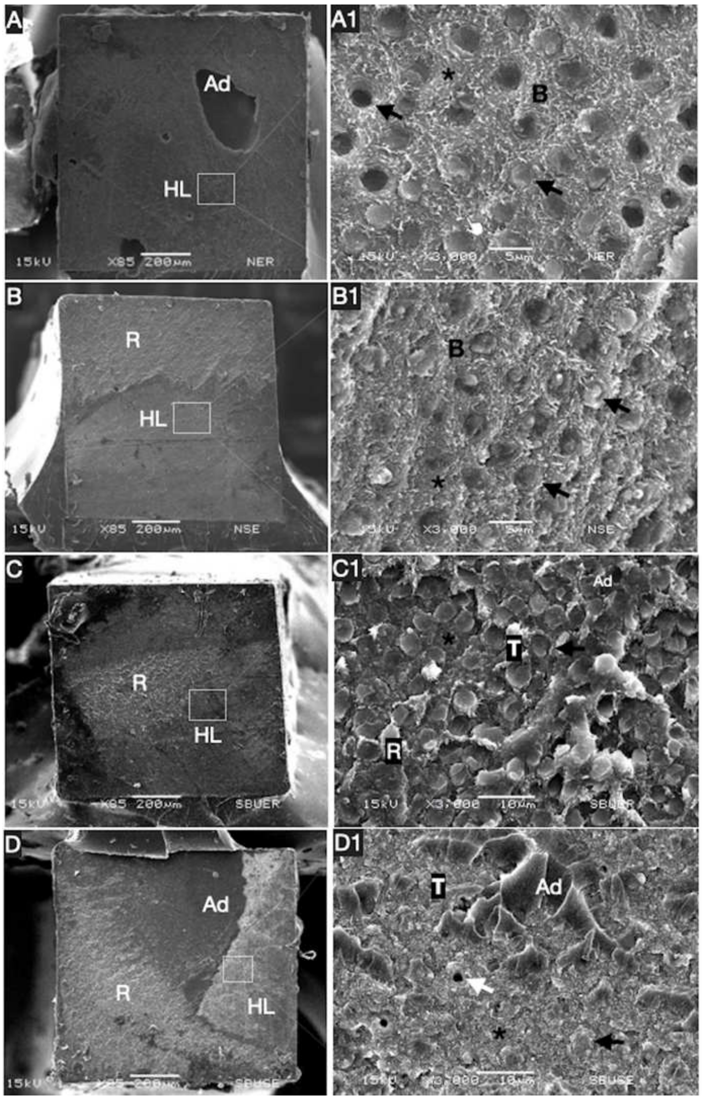

Figure 4. Representative SEM images of the fractured specimens bonded to sound dentin with the adhesives evaluated at 24 h of water storage. (A-A1) specimens bonded with SBU**P**ER; (B-B1) specimens bonded with SBU**P**SE; (C-C1) specimens bonded with SBUER; (D-D1) specimens bonded with SBUSE. Higher magnification of the area is limited by a square. (HL) Hybrid layer, (Ad) Adhesive, (R) Resin composite, (B) Bottom of the hybrid layer, (T) Top of the hybrid layer, (black arrows) dentinal tubules filled by resin tags, (asterisk) intertubular dentin covered by the adhesive; (white arrows) empty dentinal tubules. The SBU**P**ER, SBU**P**SE, SBUER (A-A1; B-B1; C-C1) showed dentinal tubules and collagen fibrils filled with the adhesive. SBUSE (D-D1) showed some dentinal tubules not filled with the adhesive.

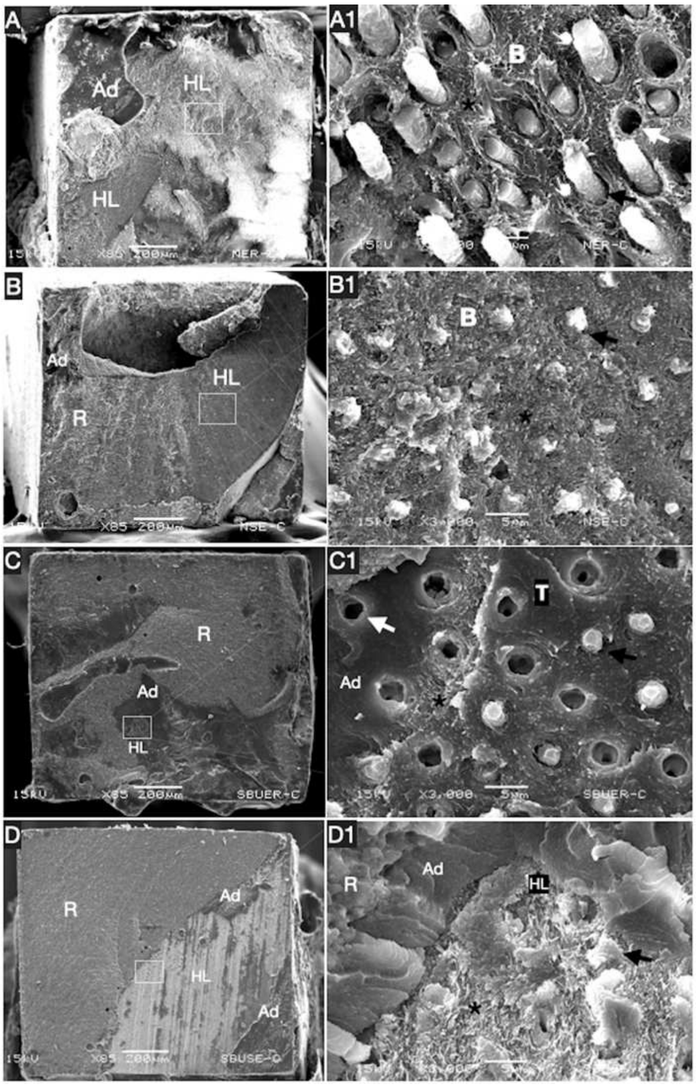

Figure 5. Representative SEM images of the fractured specimens bonded to caries-affected dentin with the adhesives tested at 24 h of water storage. (A-A1) specimens bonded with SBU**P**ER-C; (B-B1) specimens bonded with SBU**P**SE-C; (C-C1) specimens bonded with SBUER-C; (D-D1) specimens bonded with SBUSE-C. Higher magnification of the area is limited by a square. (HL) Hybrid layer, (Ad) Adhesive, (R) Resin composite, (B) Bottom of the hybrid layer, (T) Top of the hybrid layer, (black arrows) dentinal tubules filled by resin tags, (asterisk) intertubular dentin covered by the adhesive, (pointer) lack of adhesive penetration in the intertubular dentin, (white arrows) empty dentinal tubules. The prevalent failure mode was mixed failure (cohesive in the adhesive, hybrid layer, dentin, and resin composite), in which B-B1, C-C1, and D-D1 pictures showed fractures at the bottom of the hybrid layer, but resin tags did not fracture at that level A-A1 (SBU**P**ER).

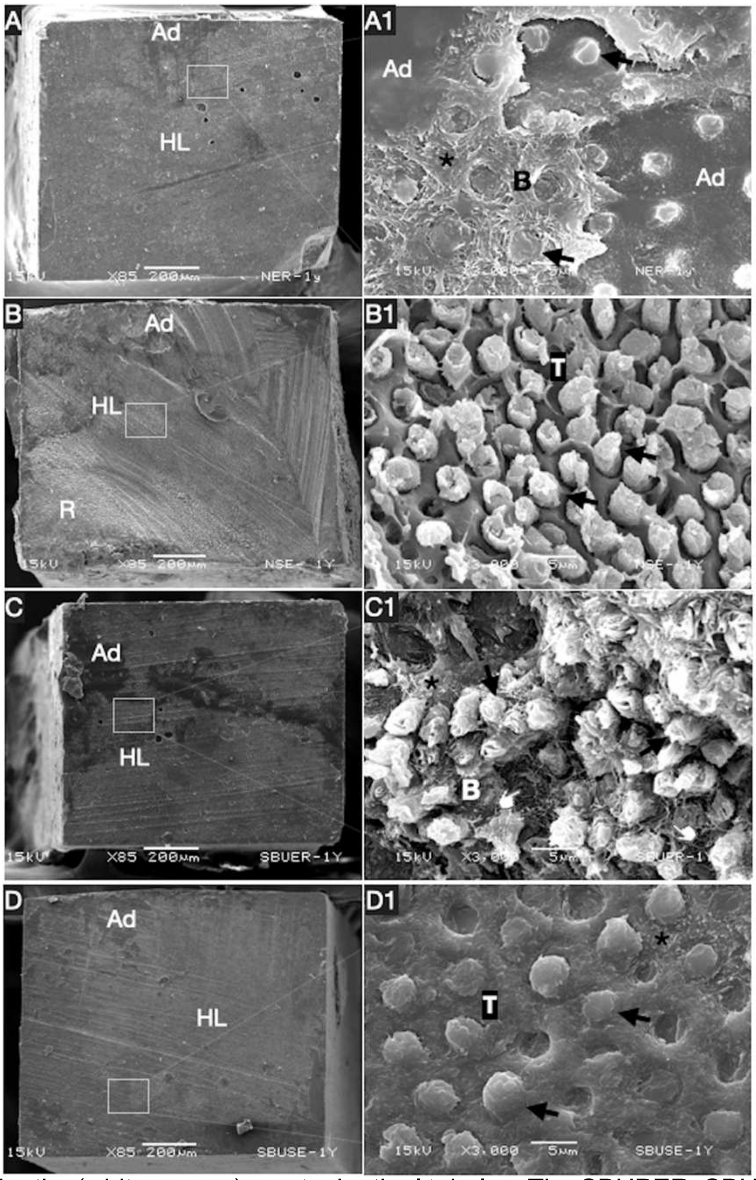

Figure 6. Representative SEM images of the fractured specimens bonded to sound dentin with the adhesives evaluated at 1-year water storage. (A-A1) specimens bonded with SBU**P**ER; (B-B1) specimens bonded with SBU**P**SE; (C-C1) specimens bonded with SBUER; (D-D1) Specimens bonded with SBUSE. Higher magnification of the area is limited by a square. (HL) Hybrid layer, (Ad) Adhesive, (R) Resin composite, (B) Bottom of the hybrid layer, (T) Top of the hybrid layer, (black arrows) dentinal tubules filled by resin tags, (asterisk) intertubular dentin covered by the adhesive, (pointer) lack of adhesive penetration in the intertubular dentin, (white arrows) empty dentinal tubules. The SBU**P**ER, SBU**P**SE, and SBUSE (A-A1; B-B1; C-C1) showed dentinal tubules and collagen fibrils filled with the adhesive. SBUER (D-D1) showed some collagen fibrils not filled with the adhesive.

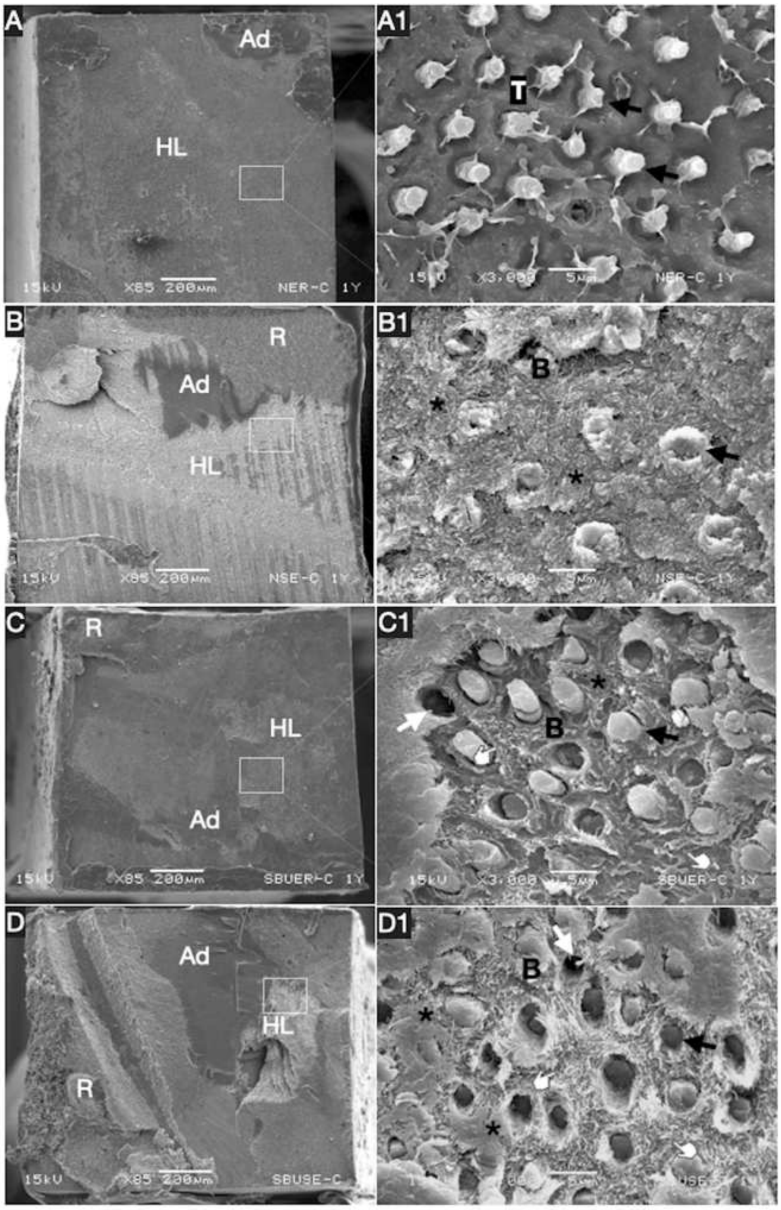

Figure 7. Representative SEM images of the fractured specimens bonded to caries-affected dentin with the adhesives tested at 1-year water storage. (A-A1) specimens bonded with SBU**P**ER-C; (B-B1) specimens bonded with SBU**P**SE-C; (C-C1) specimens bonded with SBUER-C; (D-D1) specimens bonded with SBUSE-C. Higher magnification of the area is limited by a square. (HL) Hybrid layer, (Ad) Adhesive, (R) Resin composite, (B) Bottom of the hybrid layer, (T) Top of the hybrid layer, (black arrows) dentinal tubules filled by resin tags, (asterisk) intertubular dentin covered by the adhesive, (pointer) lack of adhesive penetration in the intertubular dentin; (white arrows) empty dentinal tubules. The prevalent failure mode was the mixed failure (cohesive in the adhesive, hybrid layer, dentin, and resin composite), in which SBUER-C and SBUSE-C (C-C1, D-D1) showed fractures at the bottom of the hybrid layer with some exposed collagen fibrils and few empty dentinal tubules.

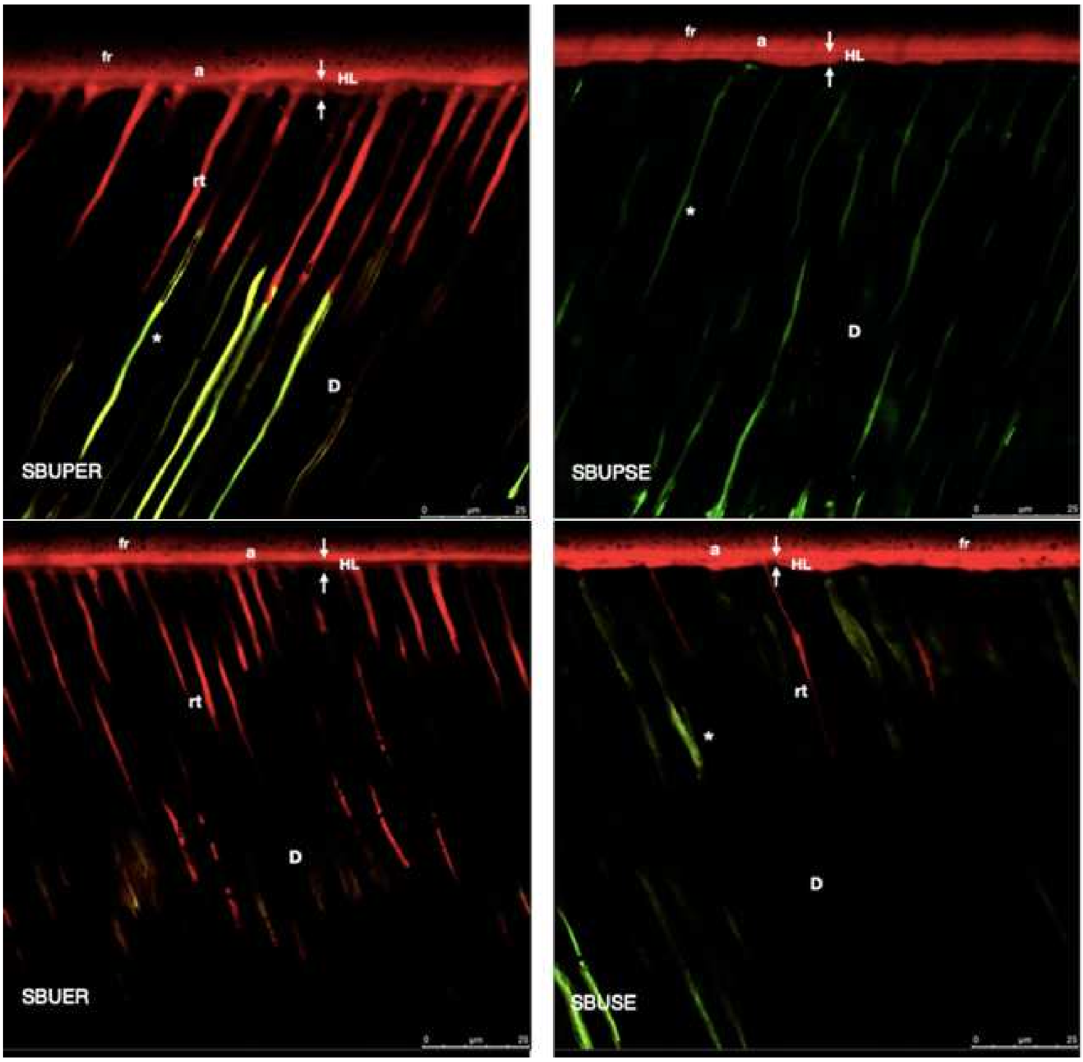

Figure 8. CLSM images illustrating the morphological characteristics of the adhesive bonding to sound dentin concerning the etch-and-rinse (ER) and self-etching (SE) application modes after 24-hour water storage. The hybrid layer (HL - between arrows) was formed by both adhesives, regardless of the application modes. The resin-dentin bonding interface for SBU**P**ER and SBUER is characterized by the long funnel-shaped resin tags (rt) underneath the adhesive layer. For the SE application modes (SBU**P**SE and SBUSE), an intensively reflective signal, ranging from the bottom of the HL to the adhesive layer, indicates a full sealing of this area. For both adhesives, the fluorescein green dye diffused into the open dentinal tubules (*) below the hybrid layer, indicating a perfect sealing of the dentin surface. The sound dentin in the mineralized area displays no reflective signal (black profile). No signs of infiltration are shown for any group. SBU**P**ER = Scotchbond Universal Plus/etch-and-rinse; SBU**P**SE = Scotchbond Universal Plus/self-etching; SBUER = Scotchbond Universal/ etch-and-rinse; SBUSE =

Scotchbond Universal/self-etching; HL = hybrid layer; rt = resin tags; a= adhesive; fr = flowable resin; * = green-dyed dentinal tubules; D = dentin. Scale bar: 25 µm.

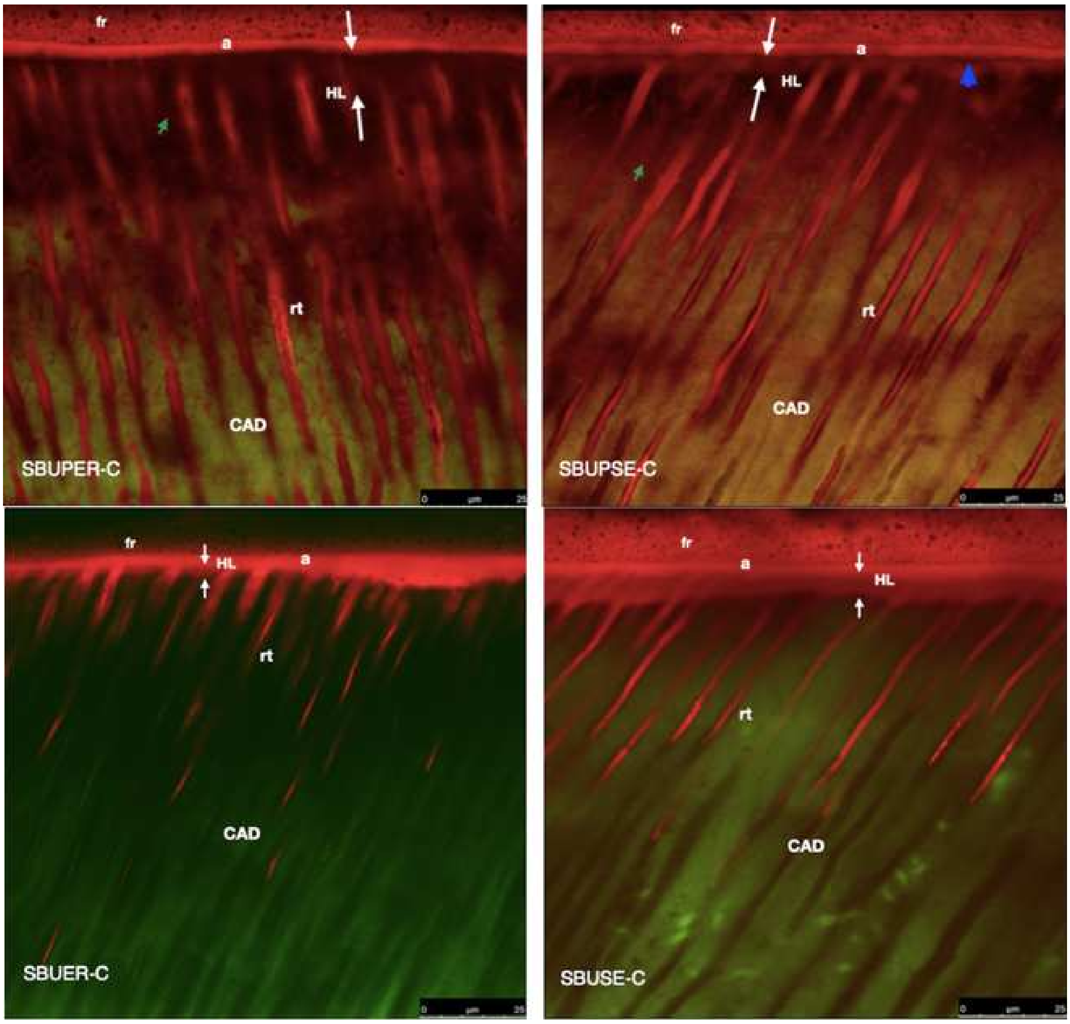

Figure 9. CLSM images illustrating the morphological characteristics of the adhesive bonding to caries-affected dentin (CAD) concerning the two application modes: etch-and-rinse (ER) and self-etching (SE) after 24-hour water storage. All resulting in a thick hybrid layer (HL - between arrows). The characteristic is represented by a slightly reflective signal from the bottom of the HL to the adhesive layer. SBU**P**ER-C and SBU**P**SE-C images show a very deep diffusion of the resin monomers in the partially demineralized tissue. The red resin tags (rt) indicate that the monomer infiltrated the dentinal tubules and their branches (green arrow). The blue arrow shows a possible technical artifact in the HL or a possible washout of the dye. The SBU**P**SE-C image shows that the resin monomer penetrates the mineralized and demineralized area in the caries-affected dentin (CAD); a spectral overlap of both dyes (red and green) resulted in an orangish/yellowish profile, which indicates the diffusion of the monomers

into the partially demineralized dentin (CAD). The resin tags (rt) underneath the adhesive layer characterize the resin-dentin bonding interface while the different intensities of the reflective green signal identify the caries-affected dentin areas for SBU**P**ER-C, SBU**P**SE-C (low), SBUSE-C (medium), and SBUER-C (high). No signs of infiltration are shown for any group. SBU**P**ER = Scotchbond Universal Plus/etch-and-rinse; SBU**P**SE = Scotchbond Universal Plus/self-etching; SBUER = Scotchbond Universal/ etch-and-rinse; SBUSE= Scotchbond Universal/self-etching; rt = resin tags; a= adhesive; fr = flowable resin; Scale bar: 25 µm.

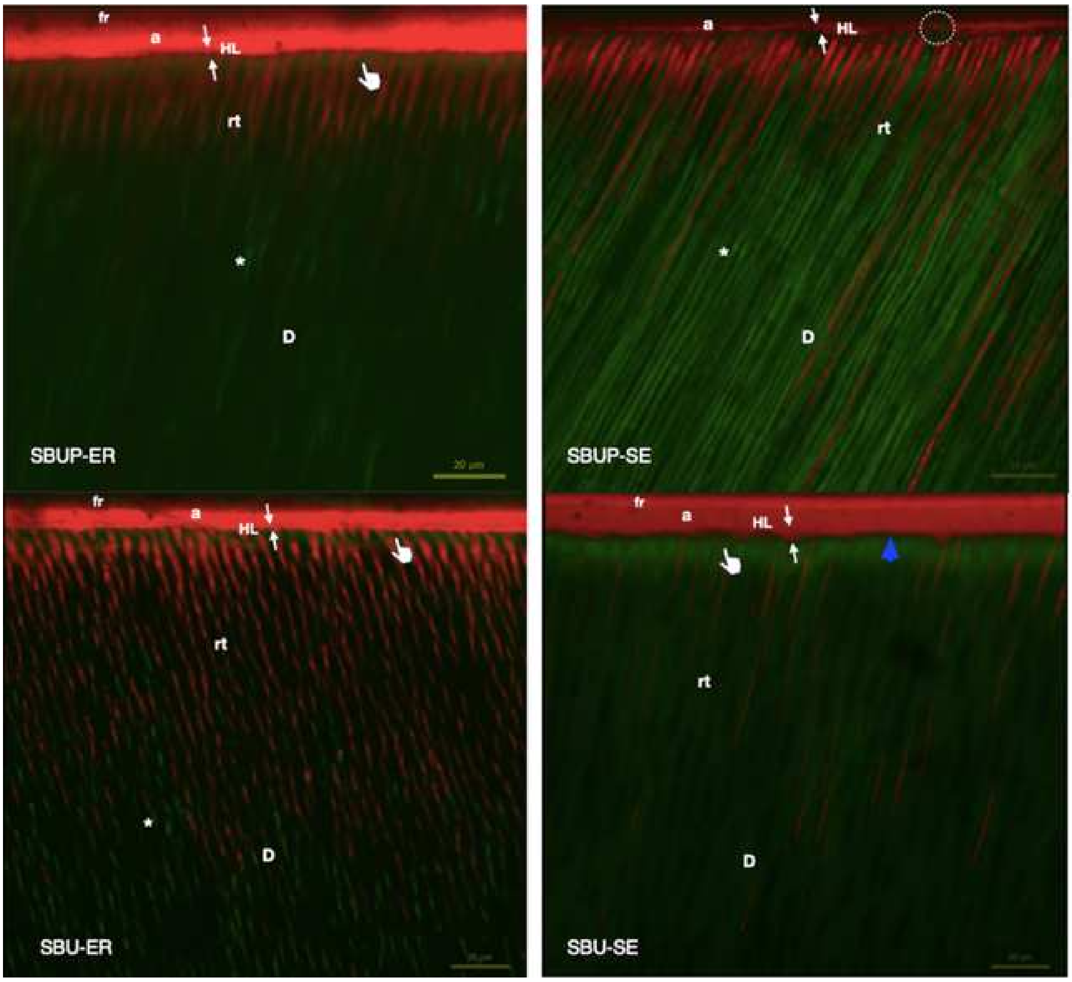

Figure 10. CLSM images illustrating the morphological characteristics of the adhesive bonding to sound dentin concerning the etch-and-rinse (ER) and self-etching (SE) application modes after 1-year water storage. Signs of infiltration (pointer) within and beneath the hybrid layer (HL) are shown in the ER mode of both adhesives. A slightly green reflective signal beneath the HL of SBUSE is shown. No infiltration of green dye was found for SBU**P** in the SE mode; although, signs of degradation are visible as an irregular HL (white circle). The blue arrow shows a possible technical artifact in the hybrid layer or a

wash out of the dye. SBU**P**ER = Scotchbond Universal Plus/etch-and-rinse; SBU**P**SE = Scotchbond Universal Plus/self-etching; SBUER = Scotchbond Universal/ etch-and-rinse; SBUSE = Scotchbond Universal/self-etching; rt = resin tags; a= adhesive; fr = flowable resin. Scale bar: 20 µm.

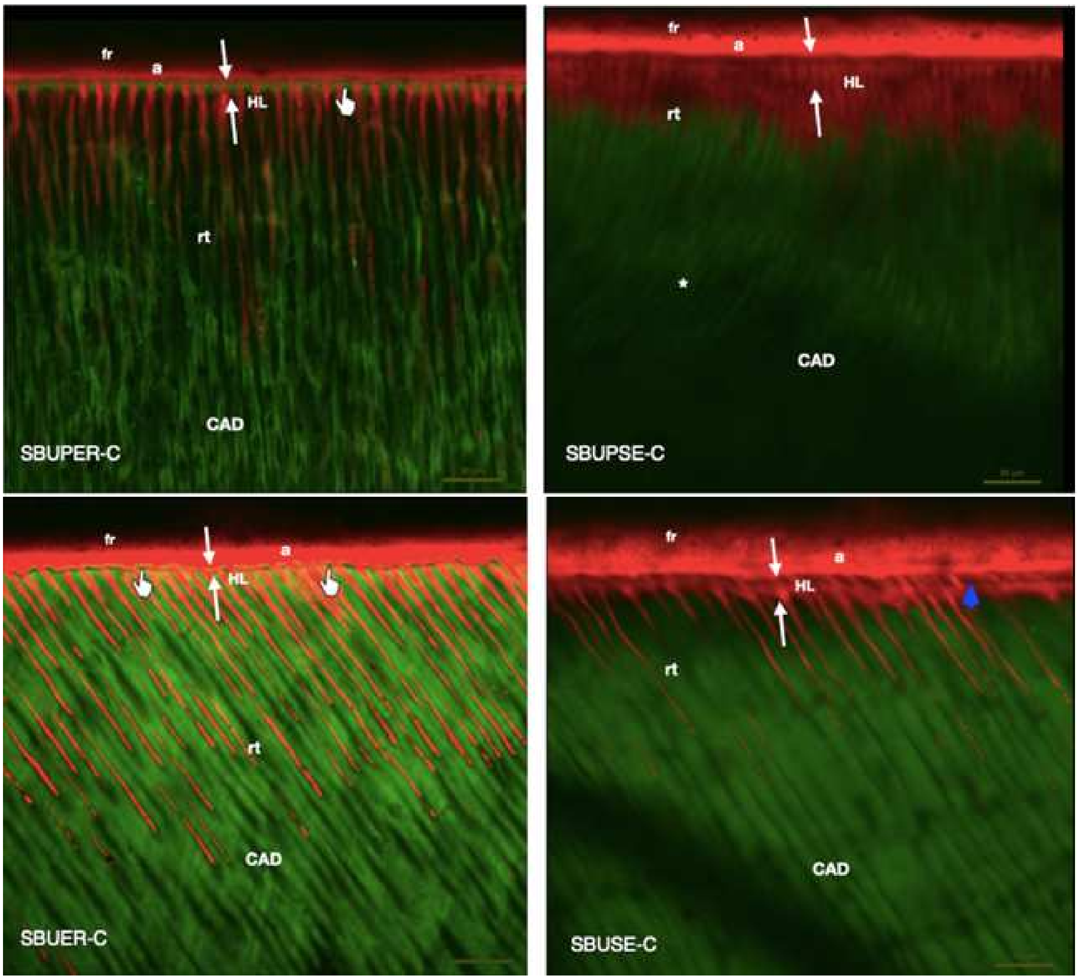

Figure 11. CLSM images illustrating the morphological characteristics of the adhesive bonding to caries-affected dentin (CAD) concerning the two application modes (ER/etch-and-rinse and SE/ self-etching) after 1-year water storage. A yellow reflective signal within and beneath the HL is shown for SBU and SBU**P** in the ER mode, which indicates water infiltration and degradation of the adhesive interface over time (pointer). No signs of infiltration are shown for the SE groups (SBU**P**SE-C, SBUSE-C). The red resin tags (rt) indicate that the monomer infiltrated the dentinal tubules and their branches. The blue arrow shows a possible technical artifact in the hybrid layer (HL) or a washout of the dye. The SBU**P**SE-C image shows that the resin monomer infiltrates the mineralized and demineralized area in the CAD; a spectral overlap of both dyes (red and green) resulted in a yellowish profile, which indicates partial dentin demineralization (CAD). The resin tags (rt) underneath the adhesive layer characterize the resin-

dentin bonding interface while the different intensities of the reflective green signal identify the CAD areas for SBU**P**ER-C (low), SBUSE-C, SBU**P**SE-C (medium) and SBUER-C (high). SBU**P**ER = Scotchbond Universal Plus/etch-and-rinse; SBU**P**SE = Scotchbond Universal Plus/self-etching; SBUER = Scotchbond Universal/ etch-and-rinse; SBUSE = Scotchbond Universal/self-etching; rt = resin tags; a= adhesive; fr = flowable resin. Scale bar: 20 μm.

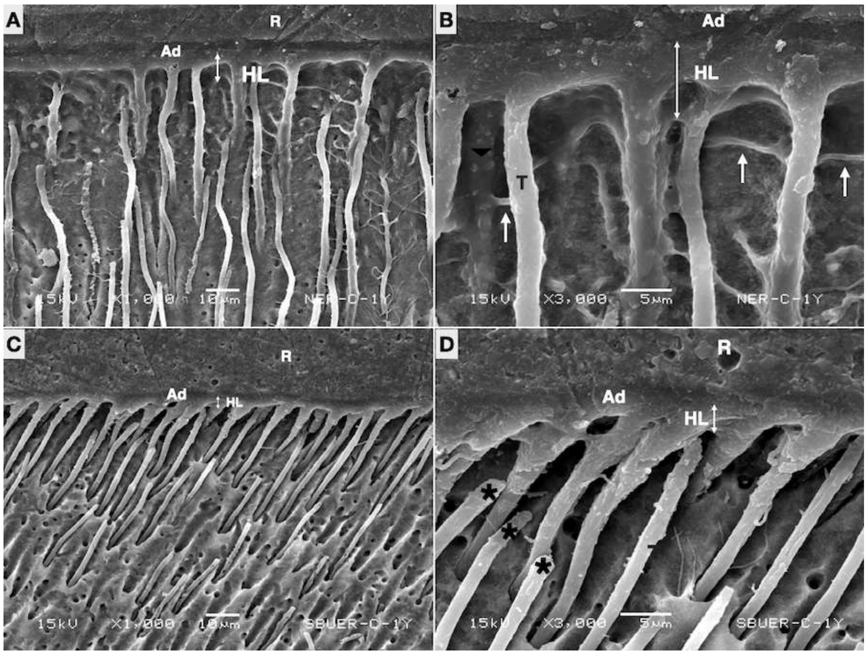

Figure 12. Photomicrographs (x1000 and x3000) obtained by SEM of SBU**P**ER-C and SBUER-C groups with 1-year water storage. SBU**P**ER applied on CAD shows deep adhesive penetration with many long resin tags with lateral branches and a thick HL (hybrid layer). Presence of globular structures which were probably formed by the grouping of small “vesicles” of polyalkenoic acid and can be observed over the resin tags (black arrow). SBU applied on CAD with the ER mode shows many resin tags along the interface some of them broken (asterisk) and a non-uniform HL. Observe in the background the porous substrate, characteristic of CAD.

TABLES

Table 1. Experimental groups according to the adhesives, application mode and dentin type

Experimental Groups	Adhesives	Application mode	Dentin
SBU**P**ER	Scotchbond Universal Plus (SBUP)	Etch-and-rinse (ER)	Sound
SBU**P**SE	Scotchbond Universal Plus (SBUP)	Self-etching (SE)	Sound
SBUER	Scotchbond Universal (SBU)	Etch-and-rinse (ER)	Sound
SBUSE	Scotchbond Universal (SBU)	Self-etching (SE)	Sound
SBU**P**ER-C	Scotchbond Universal Plus (SBUP)	Etch-and-rinse (ER)	Caries-affected dentin (C)
SBU**P**SE-C	Scotchbond Universal Plus (SBUP)	Self-etching (SE)	Caries-affected dentin (C)
SBUER-C	Scotchbond Universal (SBU)	Etch-and-rinse (ER)	Caries-affected dentin (C)
SBUSE-C	Scotchbond Universal (SBU)	Self-etching (SE)	Caries-affected dentin (C)

Table 2. Adhesives, composition, and application procedure

Adhesives/ Batch number	Composition / pH	Application Procedure
Scotchbond Universal Plus 3M/ESPE-Deutschland Gmbh, Seefeld, Germany. batch# 4897805	2-Propenoic acid, 2-methyl-, diesters with 4,6-dibromo-1,3-benzenediol 2-(2-hedroxyethoxy)ethyl 3-hydroxypropyl diethers; HEMA; 2-propenoic acid, 2-methyl-, reaction products with 1,10-decanediol and phosphorus oxide (P_2O_5) (10-MDP); water; ethanol; 2-Propenoic acid, 2-methyl-, 3-(triethoxysilyl)propyl ester, reaction products with silica and 3-(triethoxysilyl)-1-propanamine; (3-aminopropyl)triethoxysilane ; camphorquinone; copolymer of acrylic and itaconic acid; *N*,*N*-dimethylbenzocaine; acetic acid, copper(2+) salt, monohydrate; pH 3.0 or 2.7	**Self-etch (SE)**: 1. Apply the adhesive to the entire preparation with a micro-brush and rub it in for 20 s. 2. Air dry for at least 5 seconds until adhesive does not move anymore. 3. Light-cure for 10 seconds **Etch-and-rinse (ER)**: 1. Apply etchant for 15 s. Rinse thorough and air dry or cotton pellet. Do not over-dry 2. Apply adhesive as in the SE strategy.
Scotchbond Universal 3M/ESPE; St Paul, MN, USA. batch# 1933000268	HEMA; BisGMA; 2-methyl-, reaction products with 1,10-decanediol and phosphorus oxide (P_2O_5) (10-MDP); ethanol; water; 2-propenoic acid, 2-methyl-, 3-(trimethoxysilyl) propyl ester and reaction products with vitreous silica; copolymer of acrylic and itaconic acid; camphorquinone; dimethylaminobenzoat(-4); (dimethylamino)ethyl methacrylate; pH 3.0 or 2.7	**Self-etch (SE):** 1. Apply the adhesive to the entire preparation with a micro-brush and rub it in for 20 s. 2. Direct a gentle stream of air over the liquid for about 5 s until it no longer moves and the solvent is evaporated completely. 3. Light cure for 10 s. **Etch-and-rinse (ER):** 1. Apply etchant for 15 s. Rinse thoroughly and air dry or cotton pellet. Do not over-dry. 2. Apply adhesive as in the self-etch strategy.

Abbreviations: CQ: camphorquinone; HEMA: 2-hydroxyethyl methacrylate; 10MDP: 10-methacyloyloxydecyl dihydrogen phosphate; EDMAB: ethyl 4-(dimethylamino) benzoate.

Table 3. Means and standard deviations (MPa) of microtensile bond strength for all groups at different storage time (Number of sticks tested/ number of pre-failures)

	Scotchbond Universal Plus (ER)	Scotchbond Universal Plus (SE)	Scotchbond Universal (ER)	Scotchbond Universal (SE)
Sound dentin (24 h)	41.7 (6.7) Aa	43.8 (8.8) Aa	41.8 (7.9) Aa	40.2 (5.8) Aa
	(Num = 68/1)	(Num = 54/0)	(Num = 65/3)	(Num = 50/0)
Caries-affected dentin (24 h)	31.1 (7.2) Bab	32.6 (10.0) Ba	25.3 (5.8) Bb	25.98 (4.5) Bab#
	(Num = 23/2)	(Num = 28/0)	(Num = 22/3)	(Num = 20/0)
Sound dentin (1 year)	24.5 (7.8) Aa	23.4 (8.1) Aa	21.7 (9.6) Aa	24.1 (8.2) Aa
	(Num = 44/5)	(Num = 40/1)	(Num = 36/4)	(Num = 44/2)
Caries-affected dentin (1 year)	15.9 (6.0) Ba	17.7 (6.3) Aa	12.9 (6.6) Bb	20.9 (6.2) Aa#
	(Num = 18/3)	(Num = 23/2)	(Num = 17/3)	(Num = 26/3)

Different capital letters indicate statistical difference (P<0.05) in the same column of the same storage time. Sound dentin is compared to caries-affected dentin only in the same storage time.
Different lowercase letters indicate statistical difference (P<0.05) in the same row.
(#) indicates no difference between aging periods (P>0.05) for the same dentin substrate.

Table 4. Bond strength reduction in percentage between sound and caries-affected dentin for all groups at 24 hours and 1-year water storage.

	Bond strength reduction (24 h)	Bond strength reduction (1 y)
Scotchbond Universal Plus (ER)	25%	35%
Scotchbond Universal Plus (SE)	25%	25%
Scotchbond Universal (ER)	40%	41%
Scotchbond Universal (SE)	36%	14%

3. DISCUSSÃO

No cenário clínico real, o dentista frequentemente se depara com dentina afetada pela cárie, que é parcialmente desmineralizada e não requer remoção. No presente estudo, os adesivos à base de Bis-GMA (SBU) e sem BPA (SBU**P**) mostraram valores médios de µTBS significativamente maiores e melhores características morfológicas para a dentina sadia. A dentina afetada pela cárie mostrou menores valores médios de µTBS e exibiu defeitos na interface adesiva quando usado no modo 'condicionar e lavar' com o adesivo SBU. Com base nesses resultados, a primeira e a segunda hipóteses nulas foram rejeitadas.

O modo 'condicionar e lavar' quando comparado com o de autocondicionamento, desmineraliza em profundidade a dentina e expõe as fibrilas de colágeno em maior extensão (Nakajima M, et al., 1995, Nakajima M, et al, 1999, Nakajima et al, 2000), uma condição que produz infiltração adesiva incompleta (Nakajima M, et al.,1995). Esses achados estão de acordo com os observados no presente estudo, no qual a dentina afetada pela cárie no modo ER mostrou túbulos dentinários vazios e matriz de colágeno exposta (MEV), especialmente para o adesivo SBU (Figs. 5C–C1 e 7C–C1). Além disso, a aplicação do SBU no modo ER na dentina sadia e afetada pela cárie apresentou sinais de hibridização de baixa qualidade quando avaliada em microscopia confocal, caracterizada como um perfil amarelado abaixo da camada híbrida, indicando locais de defeitos interfaciais e selamento incompleto (Fig. 11).

Esses resultados podem estar associados ao maior número de falhas pré-teste, à maior infiltração de fluoresceína (1 ano) e aos menores valores médios de µTBS observados para SBU no modo ER na DAC (Tabela 3). As alterações nas características químicas e morfológicas da dentina afetada pela cárie após o condicionamento podem ser responsáveis por esses baixos valores médios de resistência de união. A DAC, parcialmente desmineralizada, mostrou uma camada híbrida irregular e mais espessa, enriquecida com componentes orgânicos (Figs. 9 e 12); esse resultado está de acordo com aqueles relatados anteriormente (Nakajima M, et al., 1995, Nakajima M, et al, 1999, Innes NP, et al., 2016, Yoshihara K, et al., 2013, Hanabusa M, et al., 2013).

O adesivo SBUP no modo ER apresentou uma difusão profunda do monômero na dentina afetada pela cárie com camadas híbridas mais espessas e tags de resina mais longos e numerosos do que aqueles observados para o SBUER (Fig. 9). Tal característica é representada por um sinal levemente reflexivo (MCVL) da parte inferior da camada híbrida para a camada adesiva. Os *tags* de resina avermelhados indicam que o monômero adesivo infiltrou os túbulos dentinários e ramificaçoes, atingindo a dentina afetada pela cárie. Os tags de resina em forma de funil abaixo da camada adesiva caracterizam uma interface de união resina-dentina selada, enquanto o sinal verde altamente reflexivo identifica a área de dentina afetada pela cárie (Figs. 9 e 11).

O valor médio de µTBS (24 h) foi menor na dentina afetada pela cárie independentemente do modo de aplicação (Tabela 3). Embora o adesivo (SBU**P**) esteja ocupando uma grande área da estrutura mineral da dentina perdida após o processo de cárie, essa quantidade de infiltrado foi insuficiente para produzir um comportamento mecânico (µTBS) semelhante ao da dentina sadia, cuja estrutura é totalmente mineralizada. Apesar disso, em relação à dentina sadia e afetada pela cárie, o SBU**P** na DAC apresentou uma redução de 25% no valor médio de µTBS quando comparado com a dentina sadia (24 h), enquanto o adesivo SBU apresentou uma redução de aproximadamente 40% (Tabela 4) em ambos os modos de aplicação, sugerindo que o SBU**P** teve um comportamento mecânico (µTBS) consideravelmente melhor do que o do SBU (Tabela 4). A redução de 40% em µTBS também foi relatada em estudos anteriores usando diferentes adesivos em 24 h na dentina afetada pela cárie (Nakajima M, et al., 1995, Yoshiyama M, et.al, 2000, Ceballos L, et.al, 2003, Erhardt MCG, et.al, 2008, Yoshida Y, et al., 2001, Chen C, et al., 2015, Pashley DH, et al., 2011, Nakajima M, et al.,1999, Pereira PN, et al., 2006, Omar H, et al., 2007, Xuan W, et al., 2010).

Durante o processo de cárie, minerais são depositados nos túbulos dentinários como resposta à agressão bacteriana, uma condição que pode afetar a infiltração dos monômeros de resina (Erhardt MCG, et.al, 2008, Zanchi CH, et al., 2006, Kenshima S, et al., 2010, Wagner A, et al., 2014). No presente estudo, apesar da obstrução mineral, o SBU**P** infiltrou efetivamente os túbulos da dentina afetada pela cárie (Fig. 9). O novo monômero na composição química do SBU**P** pode ser responsável por sua maior infiltração nas áreas desmineralizadas e nos túbulos dentinários nos modos SE e ER (Fig. 9). De acordo com seu fabricante, a viscosidade do SBU**P** foi reduzida usando cargas de sílica e solventes semelhantes aos do SBU (Alam A, et al., 2024). A característica de menor viscosidade do adesivo SBU**P** em relação ao SBU (Alam A, et al., 2024), pode ter facilitado sua infiltração na dentina afetada pela cárie, conforme indicado pelos numerosos ramos laterais visíveis nas imagens MEV (Figs. 5 e 12). Essa infiltração mais profunda de SBU**P** pode ter reforçado as estruturas dentinárias afetadas pela cárie nas primeiras 24 h, aumentando o μTBS em ambos os modos de aplicação (Tabela 3).

Apesar das alterações nas características químicas e morfológicas da DAC, o adesivo SBU**P** aplicado no modo SE neste substrato (SBU**P**SE-C), apresentou valores médios de μTBS significativamente maiores do que aqueles obtidos para o SBUER-C. Este achado pode ser responsável pela prevalência do modo de fratura Tipo I (coesivo na camada híbrida) obtido para o SBU**P**SE na dentina afetada pela cárie (Fig. 2). O SBU**P**ER-C apresentou prevalência (Fig. 2) do modo de falha misto tipo IV (fratura coesiva no adesivo, camada híbrida, dentina e resina composta). As imagens SEM da fratura na parte inferior da camada híbrida mostraram que as fibrilas de colágeno não foram preenchidas com adesivo e que os tags de resina fraturaram em locais diferentes (Figs. 5 e 7). Neste caso, a maior prevalência do modo de falha tipo IV pode ser devido à característica morfológica da DAC, que é mais poroso do que a dentina sadia. Essa condição facilita a difusão dos condicionadores inorgânicos, como o ácido fosfórico que causa uma desmineralização mais profunda.

A interface de união adesiva é composta por três condições dentinárias — a matriz colágeno levemente preenchida com adesivo, totalmente exposta e parcialmente desmineralizada (Nakajima M, et al., 2011). Em uma zona desmineralizada mais profunda, o monômero de resina dificilmente infiltra totalmente e cobre a matriz de colágeno exposta da dentina (Nakajima, M et al., 2011). Essa situação foi morfologicamente visível (Figs. 5A1, 7C1, 7D1) no presente estudo, mostrando a matriz de fibras de colágeno não preenchida totalmente com adesivo. No entanto, essa característica morfológica não teve impacto no μTBS. Os menores valores de μTBS (Tabela 3) da dentina afetada pela cárie, em comparação com a dentina sadia, podem ser devidos a uma redução na resistência coesiva da dentina. Essa redução pode estar associada à alteração nas características químicas e morfológicas da dentina afetada pela cárie.

O armazenamento em água por 1 ano reduziu significativamente o μTBS dos adesivos nos dois substrato, excepto para o grupo SBUSE-C, e afetou a morfologia da interface de união quando usado o modo ER (Figs. 10 e 12), portanto, a terceira hipótese nula foi rejeitada. O adesivo SBU**P** aplicado na dentina afetada pela cárie em 24 h apresentou redução significativa nos valores médios de μTBS (Tabela 3) nos modos ER e SE e perda de integridade da interface no modo ER (Fig. 10). No armazenamento em água por 1 ano, nenhuma diferença estatística nos valores médios de μTBS foi observada entre a dentina sadia e afetada pela cárie no modo SE (Tabela 3). As imagens MCVL (Fig. 11) mostraram um selamento completo da dentina, com os monômeros de resina adesiva totalmente infiltrados na área desmineralizada da dentina afetada pela cárie; uma sobreposição espectral de ambos os corantes (vermelho e verde) resultou em um perfil amarelado, indicando desmineralização parcial da dentina (DAC).

Este novo monômero (ácido 2-propenoico, 2 metil-diésteres com 4,6-dibromo-1,3-benzenediol 2-(2-hedroxietoxi) etil 3-hidroxipropil diéteres) que compõe SBU**P** é de natureza hidrofílica e hidrofóbica (3M Scotchbond Universal Plus Adhesive, Technical product profile, 2020) Esta característica anfifílica, o peso da molécula e os grupos hidroxila em sua composição química podem aumentar sua hidrofilicidade e molhabilidade, condições que podem ser responsáveis por sua infiltração mais profunda na dentina afetada pela cárie (Fig. 4). O armazenamento de 1 ano dos espécimes em água destilada, que foi trocada semanalmente, pode ter aumentado a sorção de água pelos grupos hidrofílicos da molécula, uma condição que pode ter reduzido a resistência de união e aumentado a degradação da interface de união de ambos os adesivos em ambos os modos de aplicação (Tabela 3). A natureza hidrofílica do adesivo SBU**P**, em contraste com a maior hidrofobicidade do Bis-GMA no SBU combinado com outros componentes hidrofílicos no mesmo frasco, pode ter resultado em uma camada superficial mais hidrofóbica no SBU, potencialmente explicando a estabilidade demonstrada ao longo do tempo usando o modo SE na CAD.

A Rodamina B (corante vermelho) e fluoresceína (corante verde) foram usadas para tornar o selamento visível na interface de união adesivo/dentina (Figs. 8–11). O perfil amarelado (imagens MCVL), resultante de uma interação do corante verde com o corante vermelho na superfície adesiva, indica defeitos interfaciais ou desmineralização parcial da dentina, que são características comuns da dentina afetada pela cárie, com maior permeabilidade quando comparada à dentina sadia. Nestes locais de desmineralização da dentina (DAC), a fluoresceína (corante verde) foi absorvida em diferentes intensidades (Figs. 9 e 11). Quanto mais profunda a cavidade e maior a perda de minerais, mais intensa foi a absorção de fluoresceína. O grau de desmineralização variou entre os dentes (DAC) e gerou diferentes intensidades de sinal reflexivo (fluoresceína: corante verde). Isso pode ser devido ao processo natural de cárie adotado no presente estudo. A Figura 9 mostra diferentes intensidades do sinal verde reflexivo para SBU**P**ER-C (baixo), SBUSE-C (médio) e SBUER-C (alto). Ela também mostra uma sobreposição espectral de ambos os corantes (vermelho e verde) resultando em um perfil amarelado (SBU**P**SE-C). Este sinal reflexivo verde, independentemente de sua intensidade, indica que a dentina afetada pela cárie foi parcialmente desmineralizada.

Todas as imagens (Figs. 9 e 11) obtidas para a dentina afetada pela cárie exibiram uma linha escura uniforme na camada híbrida (seta azul). Essa linha escura pode ser mal interpretada como uma formação de uma falha na interface de união. A invés de uma falha adesiva ou um 'gap' na camada híbrida, foi identificado como um artefato técnico, ou ruptura, resultante da preparação dos espécimes, particularmente quando a seção da interface dentina-resina foi cortada transversalmente em secções de 1 mm de espessura ou quando secções foram polidas (lixa de SiC). Essa linha escura também é visível nos tags de resina (Figs. 9 e 11). Alguns tags de resina no SBU**P**ER-C estão visivelmente conectados à camada adesiva, enquanto outras parecem começar na camada híbrida (Figs. 9 e 11).

Este estudo utilizou dentes cariados humanos, o que é uma limitação devido à dificuldade de padronização de todos os espécimes. A profundidade da superfície da dentina afetada pela cárie onde trabalhamos era inevitavelmente mais profunda do que a da dentina sadia. Isso implica menor quantidade de dentina intertubular e maior volume de dentina intratubular nas superfícies DAC, e como o SBU**P** tem uma grande capacidade de infiltrar todos os túbulos dentinários, um efeito inicial (24 h) de alta micro-retenção e selamento pode ter ocorrido —explicando também os altos valores imediatos do SBU**P**. No entanto, a longevidade de uma interface adesivo/dentina depende da interação química de ambos os substratos e do selamento da interface, como evidenciado nos resultados de 1 ano de armazenamento. Finalmente, a recomendação clínica é de usar o modo auto-condicionante (SE) com os adesivos Scotchbond Universal e Scotchbond Universal Plus em dentina sadia ou afetada pela cárie.

4. CONCLUSÃO

Com base nos resultados do presente estudo *in vitro,* pode ser concluído que:

1. O adesivo Scotchbond Universal Plus (sem BPA) infiltrou-se mais profundamente nas áreas inter e intratubulares da dentina afetada pela cárie; no entanto, não resultou em maior resistência de união.
2. O uso do condicionamento ácido afetou negativamente a resistência de união e a integridade da interface da dentina afetada pela cárie.
3. O armazenamento na água reduziu significativamente a resistência de união para os adesivos e nos tipos de dentina (sadia e afetada pela cárie), exceto para o adesivo Scotchbond Universal no modo autocondicionante.

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APÊNDICE

Apêndice 1 – Remoção da dentina infectada pela cárie

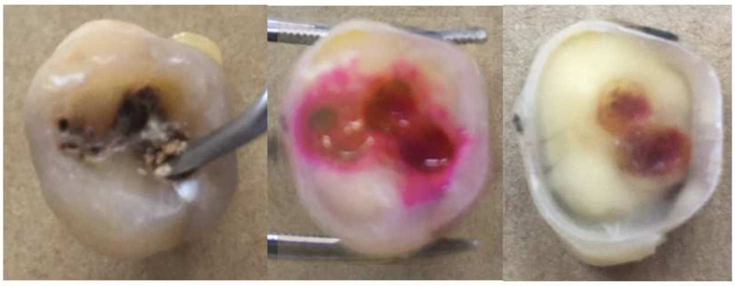

Figura. Dentina corada com detector de cárie para detecção visual. A dentina rosa-escura a vermelha foi classificada como infectada pela cárie e a dentina rosa claro como dentina afetada pela cárie (DAC).

Apêndice 2 – Microfotografías da interface adesiva obtidas por MEV

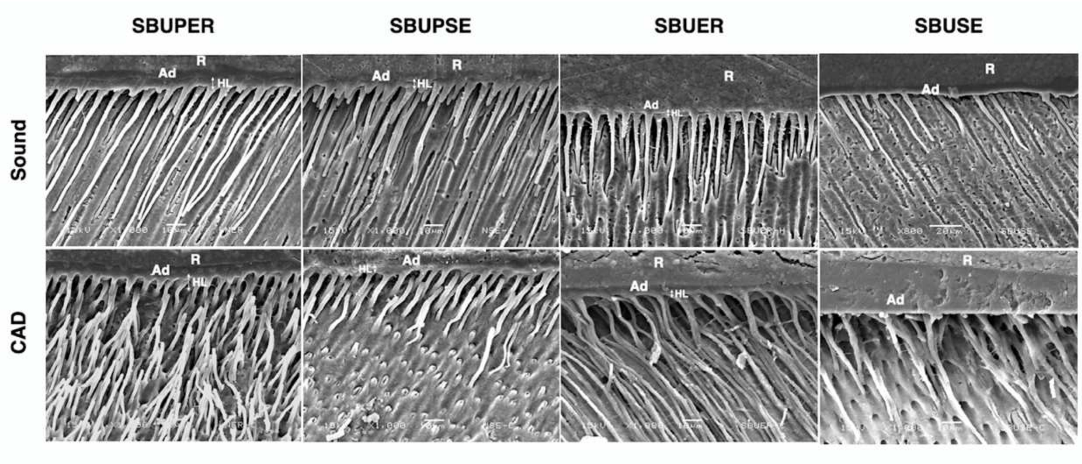

Figure. Photomicrographs (x1000) obtained by SEM for all experimental groups with 24 h water storage. Observe in SBUPER, SBUPSE and SBUER on the sound dentin the very long resin tags distributed along the interface. SBUPSE shows a great adhesive diffusion with long resin tags, longer than those of SBUSE. SBUPER on the caries-affected dentin (CAD) shows a uniform and thick hybrid layer (HL), and resin tags are displayed as multiple lateral branches along the substrate which differs from all the other groups. SBUER applied on CAD shows great adhesive infiltration where multiple disorganized resin tags with lateral branches can be observed. SBUSE applied on CAD presents less quantity of resin tags and the thinner HL when compared with the other groups. Globular structures were probably formed by the grouping of small “vesicles” of polyalkenoic acid and can be observed over the resin tags. Observe in the background the porous substrate, characteristic of CAD.

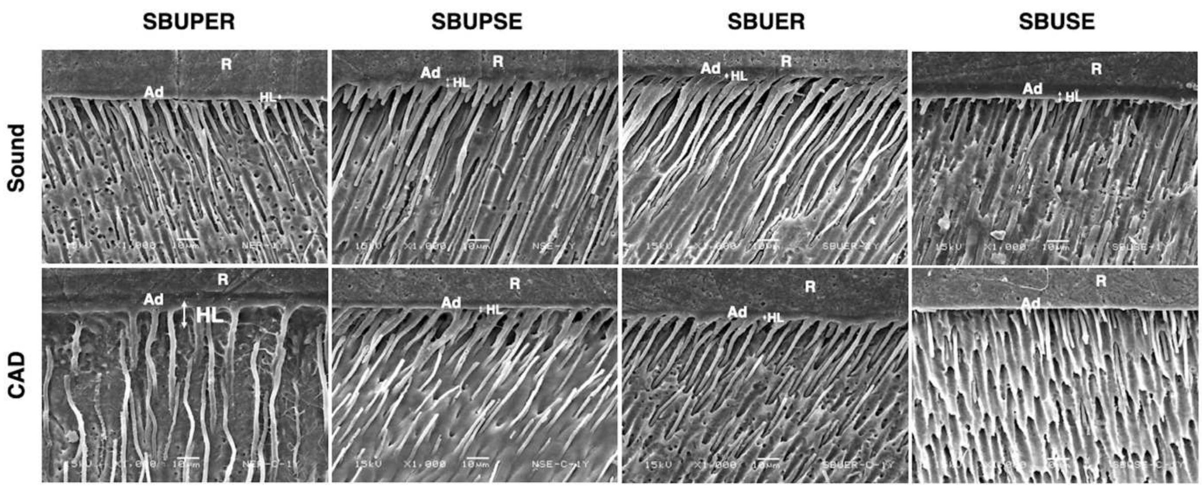

Figure. Photomicrographs (x1000) obtained by SEM of all experimental groups with 1-year water storage. SBUPER, SBUPSE, SBUER applied on sound dentin show evident long resin tags, and the integrity of the hybrid layer (HL) is maintained. SBU applied on sound dentin using the SE mode shows shorter resin tags when compared with the other groups. Presence of globular structures which were probably formed by the grouping of small “vesicles” of polyalkenoic acid and can be observed over the resin tags. SBUPER applied on CAD dentin shows great adhesive infiltration with many long resin tags with lateral branches and a thick HL. SBUP using SE on CAD presents great adhesive infiltration, and multiple long resin tags are observed. SBU applied on CAD with the ER mode shows many resin tags along the interface. SBUSE on CAD presents fewer and shorter resin tags when compared with the other groups, with some of them broken. Observe in the background the porous substrate, characteristic of CAD.

ANEXOS

Anexo 1 – Certificação do Comitê de Ética

UNICAMP - FACULDADE DE ODONTOLOGIA DE PIRACICABA DA UNIVERSIDADE DE CAMPINAS - FOP/UNICAMP

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Desempenho da união e interação morfológica de adesivos à base de MDP aplicado sobre dentina afetada pela cárie

Pesquisador: CAROLINA SANDRA GARFIAS YAMASHITA

Área Temática:

Versão: 2

CAAE: 51653621.7.0000.5418

Instituição Proponente: Faculdade de Odontologia de Piracicaba - Unicamp

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 5.048.095

UNICAMP - FACULDADE DE ODONTOLOGIA DE PIRACICABA DA UNIVERSIDADE DE CAMPINAS - FOP/UNICAMP

Continuação do Parecer: 5.048.095

Folha de Rosto	folhaDeRosto.pdf	09/09/2021 23:36:35	YAMASHITA	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

PIRACICABA, 20 de Outubro de 2021

Assinado por:
jacks jorge junior
(Coordenador(a))

Anexo 2 – Relatório de Originalidade

RELATÓRIO DE ORIGINALIDADE

6%	%	6%	%
ÍNDICE DE SEMELHANÇA	FONTES DA INTERNET	PUBLICAÇÕES	DOCUMENTOS DOS ALUNOS

FONTES PRIMÁRIAS

1	"Full Issue", Operative Dentistry, 2022 Publicação	1%
2	Luciana Andrea Salvio, Vinicius Di Hipólito, Adriano Luis Martins, Mario Fernando de Goes. "Hybridization quality and bond strength of adhesive systems according to interaction with dentin", European Journal of Dentistry, 2019 Publicação	1%
3	Andrea Wagner, Michael Wendler, Anselm Petschelt, Renan Belli, Ulrich Lohbauer. "Bonding performance of universal adhesives in different etching modes", Journal of Dentistry, 2014 Publicação	1%
4	"Full Issue PDF", Operative Dentistry, 2024 Publicação	1%
5	"Full Issue PDF", Operative Dentistry, 2023 Publicação	1%
6	Masatoshi Nakajima, Sitthikorn Kunawarote, Taweesak Prasansuttiporn, Junji Tagami. "Bonding to caries-affected dentin", Japanese Dental Science Review, 2011 Publicação	1%
7	Chenmin Yao, Mohammed H. Ahmed, Lauren De Grave, Kumiko Yoshihara et al. "Optimizing glass-ceramic bonding incorporating new	1%

Anexo 3 - Comprovante de submissão para revista científica

Ref.: Ms. No. 24-170
IN-VITRO PERFORMANCE OF A BISPHENOL A-FREE MDP-BASED ADHESIVE AND ITS INTERACTION WITH SOUND AND CARIES-AFFECTED DENTIN
Operative Dentistry

Dear Dr. De Goes,

Thank you for submitting your manuscript for publication in Operative Dentistry. The reviews raised some significant concerns. Therefore, we will not be able to accept this manuscript for publication in its current form.

Should you decide to revise the manuscript for further consideration in Operative Dentistry, your revisions should address the specific points made by each reviewer. You should also send along a cover letter, indicating your response to the review comments and any changes you have made in the manuscript.

If you choose to revise your submission, your revision is due by Jun 03, 2025.

To submit a revision, go to https://www.editorialmanager.com/jopdent/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely,

Kim Diefenderfer, DMD, MS, MS
Editor-in-Chief
Operative Dentistry

Editor's Comments:

The manuscript is well-written. It is obvious that the authors have spent considerable time on this project. However, the following modifications must be made before the manuscript can be sent for peer review:

All pages should be in Portrait orientation, rather the Landscape.
Reduce the number of Figures to < 6
References
- This Journal uses "and others" rather than "et al."
- Reference numbers should not have brackets ([]).
- Please review reference formatting style in the Guidelines to Authors.
- No punctuation in authors' names (e.g., 'de Abreu JLB' is correct. 'de Abreu J.L.B.' is incorrect).
- Each reference should have both a Volume Number and an Issue Number (if listed by the journal. Volume & Issue number should be in bold font.
- No punctuation between Volume/Issue numbers and page numbers.
- No punctuation after article title.

Operative Dentistry

IN-VITRO PERFORMANCE OF A BISPHENOL A-FREE MDP-BASED ADHESIVE AND ITS INTERACTION WITH SOUND AND CARIES-AFFECTED DENTIN

--Manuscript Draft--

Manuscript Number:	24-170R1
Article Type:	Laboratory Research
Section/Category:	
Keywords:	caries-affected dentin; longevity; Adhesion; microtensile bond strength; confocal laser scanning microscopy
Corresponding Author:	Mario Fernando De Goes, DDS PhD State University of Campinas: Universidade Estadual de Campinas Piracicaba, São Paulo BRAZIL
First Author:	Carolina Sandra Garfias, DDS, MSc, PhD student
Order of Authors:	Carolina Sandra Garfias, DDS, MSc, PhD student
	Mario Fernando De Goes, PhD
Manuscript Region of Origin:	BRAZIL
Abstract:	Purpose To evaluate the bond strength and bonding interface micromorphology of a bisphenol A-free universal adhesive in sound and caries-affected dentin, considering the etch-and-rinse and self-etching application modes and different water storage periods.