Effect of different adhesive strategies and storage time on bond strength of bi-functional monomers to simulated endodontically-treated dentin

Aline Carvalho GIROTTO[1], Gabriel ABUNA[2], Cristian SANCHEZ-PUETATE[1], Mayra Andressa PICCIONI[1], Thiago Soares PORTO[3] and Milton Carlos KUGA[1]

[1] *Department of Restorative Dentistry, School of Dentistry, São Paulo State University (UNESP), Rua Humaitá 1680, Araraquara, SP 14801-903, Brazil*

[2] *Department of General Dentistry, School of Dental Medicine, East Carolina University, 1851 MacGregor Downs Rd, Greenville NC, 27834, USA*

[3] *Division of Restorative and Prosthetic Dentistry, College of Dentistry, The Ohio State University, 305 W 12th Ave, Columbus OH, 43210, USA*

Corresponding author, Thiago Soares PORTO; E-mail: porto.14@osu.edu

Adhesive strategies were evaluated on the bond strength of bi-functional monomers bonded to endodontically-treated-dentin (ETD). Superficial dentin was removed on human molars. Teeth were immersed in 5 mL 2.5% NaOCl, followed by immersion in 5 mL 17% EDTA. Dentin surface impregnated with epoxy resin-based sealer was then divided four groups (n=10): Scotchbond Multi-Purpose (SBMP); Single Bond Universal (SBU); Optibond All-in-One (OPB); and Tetric-N-Bond Universal (TBU). After 24-h or 1-year-of-storage specimens were submitted to microtensile bond strength (μTBS) and failure classification. A confocal laser scanning microscope (CLSM) evaluated the hybrid layer formation. Two-way ANOVA and Tukey-HSD test were performed (α=5%). The μTBS did not present statistical differences among adhesive strategies after 24-h. Significant differences were found after 1-year-of-storage. CLSM analysis showed water infiltration and consequently degradation of the hybrid layer after 1-year-of-storage. The use of SBU universal adhesive on the self-etching mode on ETD produced more stable bond over the 1-year-of-storage.

Keywords: Adhesives, Endodontically-treated-dentin, Self-etching, Microtensile

INTRODUCTION

Catastrophic failure can be considered imminent in endodontically treated teeth due to loss of tooth structure, altered physical properties of dentin, and modified proprioception/nociception[1-4]. The teeth is weakened and susceptible to fracture as a result of root canal preparation, which will remove supportive and important structures. Moreover, the solutions used to perform the endodontic treatment will also contribute to disruption in the teeth structures. The organic-mineral bond of dentin inside the root canal is affected by the use of soultions such as sodium hypochlorite (NaOCl), which possess antibacterial properties and capacity for denaturation of organic proteins[1,3-5]; and EDTA a chelating agent used to treat the remaining smear layer on the radicular walls, thus facilitating the endodontic instrumentation[3,4,6]. Additionally, the modified proprioception/nociception is associated with the removal of the pulp nerve, such procedure is necessary in order to appropriately perform endodontic therapy. As result the tooth will lost sense of position and location easily susceptible to external threats. In order to increase; however, the resistance of the tooth to these threats post-endodontic restoration is indicated[7-10].

Endodontic therapy primary goal is to seal the root canal dentin in order to prevent microleakage and consequently bacterial invasion[6,10-12]. Hence, endodontic instrumentation produce smear layer and smear plugs covering so the dentin surface[13]. The use of NaOCl and EDTA is a clinical step that has been employed due to antibacterial properties and cleaning procedures of smear layer and smear plugs[5,11,14]. The endodontic sealers now on smear layer-free surface can penetrate into the dentinal tubules[3,15]. In contrast, the same solutions can induce adverse effects due to residual oxygen, a byproduct of both irrigants[2,3,16]. That may inhibit polymerization of methacrylate based materials. In addition, greater inhibition has been reported specially when total etching technique is employed[2]. Once the endodontic sealer is removed and the root canal is cleaned adhesive procedures are employed to the intra canal dentin prior to restorations[2,11,12,14,17-19].

The restoration of these teeth subjected to endodontic therapy will enhance their capability of resisting to dynamic loading in the oral environment[7-10]. Notwithstanding, the already degraded organic-mineral bond of the dentin intra-canal have to be prepared for adhesive procedures[12,20-24]. The application of the adhesive technique three-step etch-and-rinse (commonly with 37% phosphoric acid gel) will remove the remaining minerals (calcium phosphate and hydroxyapatite mainly) exposing the collagen fibrils[2,17,19,22,23,25-27]. This is a sensitivity technique once is heavily dependent on clinicians training and technical skills[21,28-33]. Primarily relying on an adequate moisture of dentin to prevent collagen collapsing[21,25,32,33]. Additionally, when an acetone-based adhesive is used it becomes highly techinique-sensitive and wet-bonding technique is mandatory[24,31-34]. The acid etching on dentin has

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been reported in the literature as totally depriving hydroxyapatite and nearly all calcium phosphates are removed[20,29,32,35-37]. This condition will rely primary bonding mechanics dependent on infiltration of adhesives to dentin *via* monomers. Besides, these monomers have weak affinity to low content of hydroxyapatite[30,35,38-42]. This process is purely based on micro-mechanical retention and almost no chemical interaction is happening.

Nonetheless, universal adhesives have been developed with different chemicals such as 10-methacryloyloxydecyl dihydrogen (10-MDP), glycerophosphate dimethacrylate (GPDM), and methacrylate carboxylic acid polymer (MCAP), which are acidic (bi-) functional monomers[19,28,29,38,43,44]. The simplified strategies by employing these functional monomers have not yet been extensively studied in endodontic treated dentin. In addition, it is reported in the literature the functional monomers with potential to chemical bonding to calcium in hydroxyapatite[28,29,40,44-46]. Even so, the acidic monomers will only partially demineralized the smear layer forming a thinner hybrid layer of about 0.3–2 μm in comparison with the 5–15 μm formed by the three-step etch-and-rinse technique[32,47]. A question arises; how these universal adhesives with acidic bi-functional monomers would perform on dentin with deprived organic-mineral content due to endodontic therapy?

Therefore, the purpose of this study was to evaluate the effect of different adhesive strategies, hybrid layer formation, and storage time on the influence of the use of bi-functional monomers to simulated endodontically-treated dentin (ETD). The null hypostheses tested were that (I) would be no difference on the bond strength and hybrid layer formation among the two adhesive strategies employed to ETD; and (II) would be no decrease on the bond strength after a period of time of the adhesive strategies used.

MATERIALS AND METHODS

This study was approved by the Research Ethics Committee of FOAr-UNESP (3.743.500) and certify that the study was performed in accordance with the ethical standards as stated in the 1964 Declaration of Helsinki and its later amendments.

Forty cavity-free human maxillary third molars were selected after the study was approved by the Research Ethics Committee (CEP) of the local university. The teeth were cleaned and stored in 1% chloramine T at 4°C. The enamel and superficial dentin of the teeth were removed to expose the deep dentin [3 mm above the cement-enamel junction (CEJ)] using a diamond disc at low-speed cutting machine (Isomet 1000, Buehler, Lake Bluff, IL, USA) under constant water irrigation. The exposed dentin surfaces were polished using 600-grit SiC paper for 60 s under running water to reproduce standardized smear layer. The dentin on the specimens were treated in order to simulate clinical conditions during endodontic treatment as follows: the specimens were individually immersed in 5 mL 2.5% NaOCl for 30 min, with the solution renewed every 3 min. Then, specimens were immersed in 5 mL 17% EDTA (Biodinâmica Ind, Ibiporã, Brazil) for additional 3 min followed by final washing with distilled water for 2 min[1-3]. Specimens were dried using absorbent paper. After this initial preparation, epoxy resin-based sealer (AH Plus, Dentsply Sirona, Konstanz, Germany) was mixed in 1:1 ratio of paste A and B, according to manufacturer instructions. The mixture was spread on the dentin surface using microbrush until visible sealer could be observed. The epoxy resin-based sealer was left undisturbed on the dentin surface of each specimen for 5 min. Finally, the dentin surface was cleaned using cotton pellet saturated with 95% ethanol until the surface appeared visibly clean.

After the initial preparation procedures, the specimens were randomly selected by lottery and divided in four groups (n=10) according to the adhesive system used. Scotchbond Multi-Purpose (SBMP; 3-step-etch-and-rinse, 3M ESPE, St. Paul, MN, USA); Single Bond Universal (SBU; self-etch mode, 3M ESPE); Optibond All-in-One (OPB; self-etch mode, Kerr, Orange, CA, USA); Tetric-N-Bond Universal (TBU; self-etch mode, Ivoclar-Vivadent, Schaan, Liechtenstein). The adhesives were applied to the dentin surface and polymerized for 10 s using a light-emitting-diode unit (LED) set to 1,200 mW/cm^2 (VALO, Ultradent Products, South Jordan, UT, USA). The irradiance on the LED unit utilized was confirmed with the use of spectrophotometer (Horiba Fluorolog 3, Horiba, Kyoto, Japan). Compositions and application mode strategy as per manufacturer instructions are listed in Table 1. After the bond procedures, all teeth received a composite restoration. Around the forty human maxillary third molars a metal tofflemire matrix (Waterpik Original Tofflemire) was stabilized with green compound (Impression compound, Kerr) to receive the composite restorations. A resin build up were made using recommended resin-based composite[48]. For SBMP (Filtek Z350XT, 3M ESPE) two increments of 2 mm were built and polymerized for 20 s with 1,200 mW/cm^2. For SBU (Filtek One Bulk Fill, 3M ESPE); OPB (SonicFill, Kerr); and TBU (Tetric-N-Ceram Bulk-fill, Ivoclar-Vivadent) a single increment of 4 mm each polymerized for 20 s with 1,200 mW/cm^2. The polymerization method for the composite resin materials followed manufacturers instruction and ISO 4049:2019 standard[49].

The specimens were stored in distilled water for 24 h at 37° C. Subsequently, specimens were longitudinally sectioned in mesial-distal and buccal-lingual directions across the bonded interface using the same low-speed diamond saw cutting device under runnning water initially used for cutting the teeth. Approximately, the obtained resin-dentin sticks had a cross-sectional area of 1 mm^2 and length of 10 mm. The measurements were confirmed with the use of a digital caliper (Digimatic Caliper, Mitutoyo, Tokyo, Japan). The split tooth apporach was used to test two times: 24 h and 1 year of water storage (37°C) on average of 15 stick per teeth

Table 1 Adhesive system/commercial name (manufacturer – batch number), composition, and application mode

Adhesive system (Manufacturer)	Composition[a]	Application mode[b]
Scotchbond Multi-Purpose (3M ESPE) 3 step etch-and-rinse adhesive system pH primer 2.9–4 pH adhesive —no data available batch: N546898	Universal etchant: water, phosphoric acid, synthetic amorphous silica, polyethylene glycol, aluminum oxide Primer: water, HEMA, copolymer of acrylic and itaconic acids Bond: Bis-GMA, HEMA	Etch-and-rinse strategy 1. Acid etching with 37% phosphoric acid (15 s), rinsing (15 s) and dry with cotton pellets leaving dentin surface moist; 2. Application of 2 coats of primer; 3. Gently air-dried (5 s at 20 cm); 4. Application of the adhesive; 5. Light-cure for 10 s at 1,200 mW/cm^2.
Single Bond Universal Adhesive (3M ESPE) Universal adhesive pH 2.7 batch: 16057004427	10-MDP, HEMA, Bis-GMA, 2-propenoic acid, 2-methyl-reaction products with 1,10-decanediol and phosphorous oxide, ethanol, water, 2-propenoic acid, 2-methyl- 3-(trimethoxysilyl) propyl ester, reaction products with vitreous silica, copolymer of acrylic and itaconic acid, CQ, dimethylaminobenzoat(4-), (dimethylamino)ethyl methacrylate	Self-etch strategy 1. Dentin surface washed with air/water spray; 2. Gently air-dried (5 s at 20 cm) 3. Adhesive was applied to the entire surface with a micro-brush and rubbed for 20 s; 2. Direct gentle stream of air over the adhesive layer for 5 s until solvent has evaporated completely; 3. Light-cure for 10 s at 1,200 mW/cm^2.
OptiBond All-In-One (Kerr) 1 step self-etch adhesive system (All-in-one) pH 2.5 batch: 5850929	GPDM, HEMA, Glycerol dimethacrylate, Bis-GMA, water, acetone, ethyl alcohol, CQ, nanosilica and sodium hexafluorosilicate fillers	Self-etch strategy 1. Dentin surface washed with air/water spray; 2. Adhesive was applied onto the surface with a brushing motion for 20 s (two layers); 3. Direct gentle stream of air over the adhesive layer for 5 s until solvent has evaporated completely; 4. Light-cure for 10 s at 1,200 mW/cm^2.
Tetric N- Bond Universal (Ivoclar-Vivadent) Universal adhesive pH 2.5–3.0 batch: T34374	10-MDP, HEMA, Bis-GMA, D3MA, MCAP, ethanol, water, highly dispersed silicon dioxide and CQ	1. Dentin surface washed with air/water spray; 2. Adhesive was applied onto the surface with a brushing motion for 20 s (two layers); 3. Direct gentle stream of air over the adhesive layer for 5 s until solvent has evaporated completely; 4. Light-cure for 10 s at 1,200 mW/cm^2.

[a]HEMA, 2-hydroxyethyl methacrylate; Bis-GMA, bisphenol A diglycidyl ether dimethacrylate; 10-MDP, 10-methacryloyloxydecyl dihydrogen phosphate; GPDM, glycerol phosphate dimethacrylate; MCAP, methacrylated carboxylic acid polymer; D3MA, 1,10 decandiol dimethacrylate; CQ, camphorquinone.
[b]According to the manufacturer's instructions.

tested. The distilled water for 1 year of storage was weekly renewed and neither antibiotics nor antifungals were used. For microtensile bond strength (μTBS) testing, the resin-dentin sticks were fixed to a testing jig with cyanoacrylate glue (Super Bonder gel, Loctite Henkel, Rocky Hill, CT, USA) and tested in a universal testing machine (EZ-test, Shimadzu, Kyoto, Japan) with a 5-kN loading cell at a crosshead speed of 1 mm/min. The μTBS values (MPa) were calculated by dividing the load at complete failure by the cross-sectional bonding area: $\mu TBS = F/a * 0.981$. Where, μTBS is bond strength in Mpa; F is load required to fracture the specimen in kgF; a is the bond interface area in mm^2; and 0.981 transformation constant unit from kgF/cm^2 to Mpa. The failure mode for each specimen was scrutinized using an optical microscope at 60× magnification (M80, Leica Microsystems, Heerbrugg, Switzerland), then classified as as follows: cohesive failure in dentin, cohesive failure in composite; adhesive failure between composite and dentin; and mixed failure (the three structures were involved).

Three teeth per group were prepared for observation under a confocal laser scanning microscope (CLSM). Each of the three specimens were longitudinally divided in halves (analyses after 24 h and 1 year of storage), and both surfaces were wet-polished for 1 min with 800-,

1200-, and 2000-grit SiC paper. The halves of resin-dentin sticks were restored using the same adhesive doped with 0.5% of Rodhamine B (Sigma Aldrich, St. Louis, MO, USA) immersed for 3 h prior to observation in a solution of sodium fluorescein 100 mM (Sigma Aldrich) using Leica SP5 TCS confocal laser scanning microscope (Leica Microsystems, Hanheim, Germany). The specimens were assessed using Ex/Em of 488/520 and 561/594 with 20 μm of Z-stack at 0.2 μm step size using an immersion objective 63× NA1.4.

To the data obtained for μTBS, Shapiro-Wilk and Levene test were employed for correct assumptions of normality and homogeneity of variances. Two-way ANOVA and Tukey *post-hoc* test were carried out at a significance level of 5%. The software Statistical Package for the Social Sciences (SPSS 25.0, IBM Software Solutions, New York, NY, USA) was used for statistical analysis.

RESULTS

The μTBS results are summarized in Table 2. Table 3 presents the two-way ANOVA statistics. The μTBS after 24 h did not show statistically significant differences among the adhesive systems tested ($p>0.05$). However, significant differences were found after 1 year of storage compared to 24 h for SBMP ($p<0.05$), OPB ($p<0.05$), and TBU ($p<0.05$). There was one exception to SBU after 1 year, which did not present decrease in μTBS compared to 24 h. Consequently, SBU was not statistically significant compared before and after storage ($p=0.068$). The universal adhesive systems SBU and TBU were statistically different from each other compared after 1 year of storage ($p<0.05$). Conversely, SBU was not statistically significant compared to SBMP (3-step-etch-and-rinse) and OPB (self-etching mode) ($p>0.05$).

The complete pattern of failure modes are showed in Fig. 1. SBMP, SBU, and TBU increased the adhesive failure after one year of storage. Conversely, OPB showed less adhesives failures after one 1 year compared to 24 h of storage. OPB cohesive failures increased either in resin or in dentin.

Differences were observed in the thickness of the hybrid layer. Figures 2A and 2B, which represents SBMP showed the thickest hybrid layer. SBMP after 1 year of storage evidenced presence of water in the resin tags as seen in Fig. 2B. SBU, OPB, and TBU are seen in Figs. 3A, 3B, 4A. 4B, 5A, and 5B. They all showed thinner hybrid layer than SBMP and of similar thickness among them, which is appropriate to self-etching mode adhesives. SBU showed uniform formation of resin tags after 24 h evaluation as seen in Fig. 3A. Even though SBU showed the longest formation of resin tags, which could be attributed to increased mean of μTBS after one year of storage, discontinuity on the resin tags were observed in Fig. 3B. OPB showed air trapped (porous areas) inside the hybrid layer after 24 h of storage as seen in Fig. 4A, which could explain the presence of water in the hybrid layer after 1 year of storage evidenced by Fig. 4B. TBU did not present a uniform resin tag formation after 24 h of storage as seen in Fig. 5A, similar condition was observed after 1 year of storage as seen in Fig. 5B, which could have reflected on the low μTBS and clear discontinuity of the resin tags.

Table 2 Means and standard deviations (SD) of μTBS (MPa) results after 24-h and 1-year-storage

Adhesives	24-h	1-year-storage
SBMP	51.40 (9.82)^{Aa}	39.66 (6.94)^{Ba}
SBU	54.03 (10.21)^{Aa}	45.35 (8.06)^{Aa}
OPB	54.42(13.59)^{Aa}	41.73 (3.18)^{Ba}
TBU	54.55 (9.83)^{Aa}	31.30 (8.67)^{Bb}

Means followed by the same letter (capital letters in the rows [storage time] and lower letters in the columns [adhesives]) indicate statistically similar values ($p>0.05$).

Table 3 Two-way ANOVA for adhesives systems, storage time, and interaction between both variables

Source	Sum of squares	df	Mean square	F	Sig.
Intercept	183,549.34	1	183,549.34	2,618.48	0.000
adhesives	406.01	3	135.34	1.93	0.131
storage	3,886.18	1	3,886.18	55.44	0.000
adhesives*storage	697.70	3	232.57	3.32	0.024
Total	194,147.04	88	—	—	—

a. R squared=0.471 (adjusted R squared=0.425)/b. computed using alpha=0.05

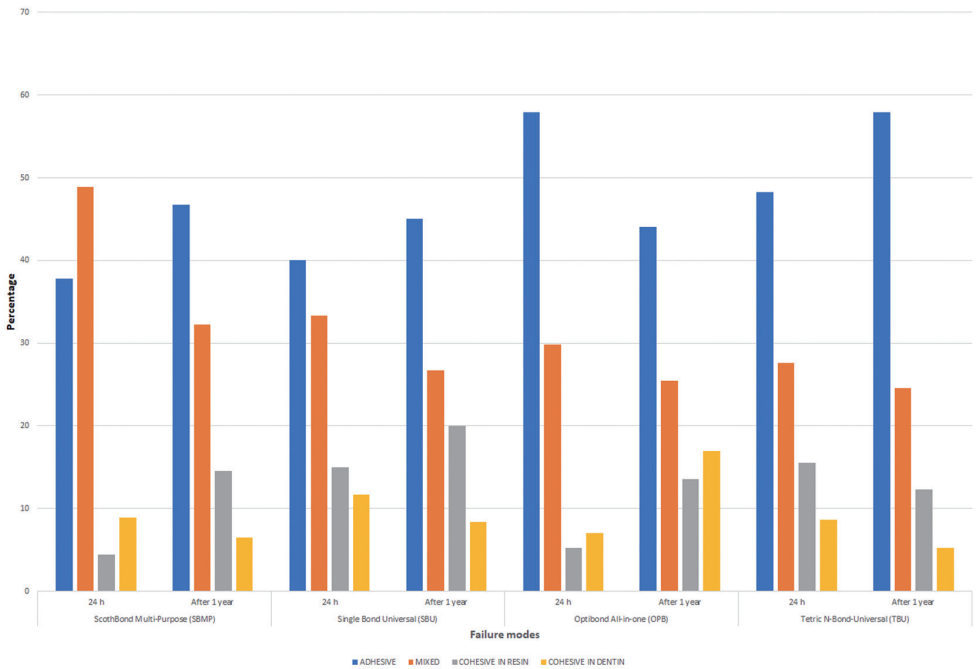

Fig. 1 Failure modes distributions according to different adhesive system groups.

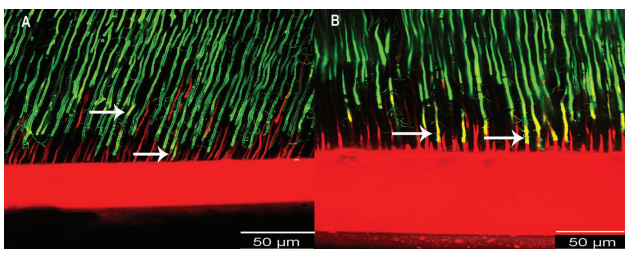

Fig. 2 Representative confocal laser scanning micrographs image of resin-dentin adhesive SBMP.
A, 24 h of storage white arrows depicted areas suggestive of water within resin tags possible remnants from 3-step etch-and-rinse technique; B, 1 year of storage white arrows identify infiltration in yellow of water within resin tags.

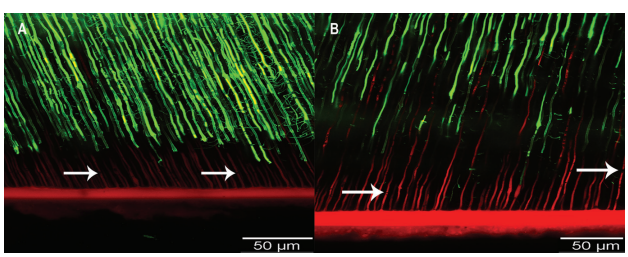

Fig. 3 Representative confocal laser scanning micrographs of resin-dentin adhesive SBU.
A, 24 h of storage white arrows shows fractured resin tags and along dentinal tubules yellows areas of water are visible; B, 1 year of storage white arrows point fractured resin tags within hybrid layer; however, no water has been identified neither within tubules nor in hybrid layer.

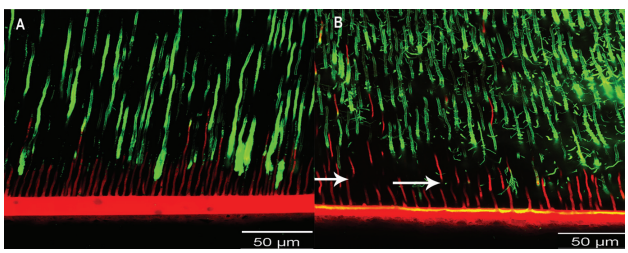

Fig. 4 Representative confocal laser scanning micrographs of resin-dentin adhesive OPB.
A, 24 h of storage no clear visible water remnants are present and resin tags look intact; B, 1 year of storage white arrows identify fractured resin tags and yellow line shows water infiltration within hybrid layer.

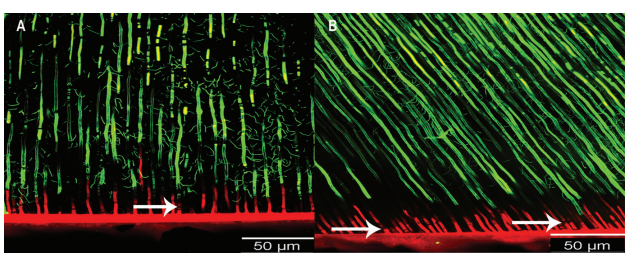

Fig. 5 Representative confocal laser scanning micrographs of resin-dentin adhesive TBU.
A, 24 h of storage white arrow shows fractured resin tag and along dentinal tubules yellows areas of water are visible; B, 1 year of storage white arrows show fractured resin tags and along dentinal tubules yellows areas of water are visible.

DISCUSSION

The present study aimed to evaluate the effect of different adhesive strategies, hybrid layer formation, and storage time on the influence of the use of bulk-fill composites to simulated ETD. In the literature is reported that adhesives with low pH may exhibit deeper penetration into ETD. This penetration is due to the increased permeability caused by degradation of collagen fibrils with the use of NaOCl and demineralization caused by EDTA30[1]. NaOCl will induce a loss in organic matrix within dentin and EDTA an additional demineralization to an already deproteinized substrate. These substances likely create a dissolution of the collagen-mineral bond associated with the smear layer that differs from those that are found in substrates without endodontic treatment[1,16,22,50]. In addition, the aggressiveness of self-etching adhesives, which depends on its own pH levels, may change the depth of interaction among these adhesives agents with ETD and consequently influence bond strength and longevity for the reasons aforementioned[13,19,26,42]. Even though the comparison among the adhesive strategies after 24 h of storage did not present statistically significant results, the comparison after 1 year of storage presented relevant findings. In addition, the hybrid layer formation evaluation on CLSM showed distinct characteristics among the different adhesive strategies employed. Thus, both null hypotheses were rejected.

Three-step etch-and-rinse adhesives are associated with deeper penetration of resin tags due to the application of 37% phosphoric acid on dentin that will complete remove the smear layer and consequently cause demineralization (5–15 μm after 15 s) of the inorganic constituent of dentin. Thus, exposing the collagen fibrils, which will be infiltrated by the hydrophilic monomers presented in the primer[20,21,26,42,43]. During the application of the 3-step etch-and-rinse system (SBMP), adequate moisture levels must be maintained to prevent the collapse of the collagen fibrils and this collapse is associated with decrease of μTBS and degradation of hybrid layer[26,42]. Depending on moisture concentrations, when the resin monomers infiltrate the demineralized collagen matrix, some regions could not be adequately filled in. The lack of adequate moisture may produce defects inside the hybrid layer[22,45]. Therefore, under these conditions water-filled niches can activate proteolytic enzymes present in the collagen matrix[24] and these enzymes trigger hydrolytic and enzymatic degradation that will negatively affect the long-term efficacy of the adhesive system (SBMP)[25]. Undoubtedly, this is the case for sound dentin[22,24,30,37,40,42]. Similarly, the exposed collagen fibrils of ETD were found to be vulnerable to hydrolytic degradation processes after 1 year of storage. Hence, the confocal microscopy images proved this phenomenon by showing water within the adhesive layer on the SBMP and OPB.

The interaction between sound dentin and self-etching adhesives is more superficial[13,26,29]. Notwithstanding, the results observed in the CLSM images within this study showed a deeper penetration of self-etching adhesives. According to the manufacturer, the pH of the adhesives used in this study ranges from 2.5 to 3 (Table 1). Therefore, the adhesives are classified as ultra-mild. Ultra-mild self-etching adhesives tend to form one thin sub-micrometric hybrid layer (<1 μm) and resin tags are infrequent[13]. The significant level of bond strength produced by SBU, OPB, and TBU after 24 h and after 1 year of storage time may be associated with substantial and different aspects. The characteristics of hybrid layer, penetration, and resin tag formation exhibited by all of the adhesives studied could be explained cause NaOCl and EDTA were applied to the deep dentin. In deep regions, the density of the tubules is known to vary from 59,000 to 76,000 per mm^2 and tubules are approximately 2.37 μm in diameter[32]. In addition, the area occupied by intertubular dentin is 12% only, which is associated with the chemical interactions produced by adhesives components[23]. In regards of the μTBS results, SBU was the only adhesive that did not present reduction in μTBS after 1 year of storage. Furthermore, SBU also differed from the SBMP and OPB adhesives where water was not found in the hybrid layer after aging. These results are explained by the presence of the functional monomer 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) in SBU composition. Relative to other monomers, currently 10-MDP is the most effective functional monomer. 10-MDP possesses a 10 units alkane hydrophobic spacer chain, which results in polarized organization that enables a stable adhesion of the phosphoric functional group to the tooth structure enhancing the bond durability to protect the hybrid layer from hydrolytic degradation[19,38,47]. As observed in previous reports[29,40,43].

In contrast to the findings on the presence of 10-MDP, the functional monomer in OPB is glycerolphosphate dimethacrylate (GPDM). A recent study concluded that the bond formed between GPDM and hydroxyapatite was weaker than the bond formed between hydroxyapatite and 10-MDP[38]. Although this adhesive contains acetone, which is a high vapor pressure solvent that contributes to long-term efficacy, the lack of formation of a stable calcium salt in the present study could have resulted in diminished μTBS after aging[38,39]. That is also supported by presence of water within the hybrid layer. Yet, it is important to indicate that even though the μTBS values for OPB have diminished with substantial difference after 1 year and water was found in hybrid layer, there were no statistical differences compared to SBMP and SBU.

TBU, which is also a universal adhesive with 10-MDP showed differences and similarities with the other studied adhesive systems. The mixture and dilution of 10-MDP with other monomers to produce new adhesive products may affect the ability of 10-MDP to provide adhesive stability[43]. Like the inhibition of nanolavering by HEMA[51], TBU has also the functional methacrylated carboxylic acid polymer (MCAP), which is also capable of reacting and bonding to hydroxyapatite. The lower concentration of its components could explain the

weaker μTBS after 1 year of storage, since 10-MDP and MCAP may have competed in their bonding capabilities with the calcium on dentin. Furthermore, differences in purity of 10-MDP from different manufacturers may result in different adhesive behaviors; however, this assumption is a limitation on this study[29,39].

Other limitation of this study was associated to identify the reason of the increased amount of adhesive failures with the OPB adhesive system. The authors hypothesized that can be associated with the technique related to the bulk-fill composite system used with this adhesive. The composite requires a hand-piece to apply sonic energy and to lower the viscosity of the material during placement. That might have influenced the failures. Further studies should be carried to investigate the influence of such equipment on the mechanical properties of this composite. In addition, fillers size-particles of each composite resin could also have influenced the μTBS results and failure modes. The sorption and solubility of composites are altered by fillers size possibly influencing the stability of bond strength.

CONCLUSIONS

Within the limitations of this *in vitro* study the following conclusions were drawn:

1. SBU universal adhesive employed as self-etching mode provided more stable bond strength on simulated ETD over the period of year storage compared to three-step etch-and-rinse.
2. Universal adhesives employed as self-etching mode presents usually thinner hybrid layer formation; however, the resin tags are comparable to three-step etch-and-rinse on simulated ETD.
3. Longer resin tags formation on ETD can be associated with increased dentin permeability due to NaOCl and EDTA and not directly with etching procedures or the pH associated with universal adhesives.
4. The functional monomer 10-MDP showed hydrophobic characteristics and substantial effect of preventing water in the hybrid layer after one year of storage.

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REFERENCES

1) Sim TP, Knowles JC, Ng YL, Shelton J, Gulabivala K. Effect of sodium hypochlorite on mechanical properties of dentine and tooth surface strain. Int Endod J 2001; 34: 120-132.
2) Vongphan N, Senawongse P, Somsiri W, Harnirattisai C. Effects of sodium ascorbate on microtensile bond strength of total-etching adhesive system to NaOCl treated dentine. J Dent 2005; 33: 689-695.
3) Wattanawongpitak N, Nakajima M, Ikeda M, Foxton RM, Tagami J. Microtensile bond strength of etch-and-rinse and self-etching adhesives to intrapulpal dentin after endodontic irrigation and setting of root canal sealer. J Adhes Dent 2009; 11: 57-64.
4) Weston CH, Ito S, Wadgaonkar B, Pashley DH. Effects of time and concentration of sodium ascorbate on reversal of NaOCl-induced reduction in bond strengths. J Endod 2007; 33: 879-881.
5) Marending M, Luder HU, Brunner TJ, Knecht S, Stark WJ, Zehnder M. Effect of sodium hypochlorite on human root dentine —mechanical, chemical and structural evaluation. Int Endod J 2007; 40: 786-793.
6) Pascon FM, Kantovitz KR, Sacramento PA, Nobre-dos-Santos M, Puppin-Rontani RM. Effect of sodium hypochlorite on dentine mechanical properties. A review. J Dent 2009; 37: 903-908.
7) Faria AC, Rodrigues RC, de Almeida Antunes RP, de Mattos MaG, Ribeiro RF. Endodontically treated teeth: Characteristics and considerations to restore them. J Prosthodont Res 2011; 55: 69-74.
8) Landys Borén D, Jonasson P, Kvist T. Long-term survival of endodontically treated teeth at a public dental specialist clinic. J Endod 2015; 41: 176-181.
9) Ozturk B, Ozer F, Belli S. An in vitro comparison of adhesive systems to seal pulp chamber walls. Int Endod J 2004; 37: 297-306.
10) Ray HA, Trope M. Periapical status of endodontically treated teeth in relation to the technical quality of the root filling and the coronal restoration. Int Endod J 1995; 28: 12-18.
11) Bohrer TC, Fontana PE, Lenzi TL, Soares FZM, Rocha RO. Can endodontic irrigating solutions influence the bond strength of adhesives to coronal dental substrates? A systematic review and meta-analysis of in vitro studies. J Adhes Dent 2018; 20: 481-494.
12) Burrer P, Dang H, Par M, Attin T, Taubock TT. Effect of over-etching and prolonged application time of a universal adhesive on dentin bond strenght. Polymers (Basel) 2020; 12: 2902.
13) Tay FR, Pashley DH. Aggressiveness of contemporary self-etching systems. I: Depth of penetration beyond dentin smear layers. Dent Mater 2001; 17: 296-308.
14) Santos JN, Carrilho MR, De Goes MF, Zaia AA, Gomes BP, Souza-Filho FJ, *et al*. Effect of chemical irrigants on the bond strength of a self-etching adhesive to pulp chamber dentin. J Endod 2006; 32: 1088-1090.
15) Bandeca MC, Kuga MC, Diniz AC, Jordão-Basso KC, Tonetto MR. Effects of the residues from the endodontic sealers on the longevity of esthetic restorations. J Contemp Dent Pract 2016; 17: 615-617.
16) Rueggeberg FA, Margeson DH. The effect of oxygen inhibition on an unfilled/filled composite system. J Dent Res 1990; 69: 1652-1658.
17) 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.
18) Mokhtari F, Anvar E, Mirshahpanah M, Hemati H, Danesh Kazemi A. The probable effect of irrigation solution and time on bond strength to coronal dentin: An in vitro evaluation. Iran Endod J 2017; 12: 439-442.
19) Saito T, Takamizawa T, Ishii R, Tsujimoto A, Hirokane E, Barkmeier WW, *et al*. Influence of application time on dentin bond performance in different etching modes of universal adhesives. Oper Dent 2020; 45: 183-195.
20) De Munck J, Mine A, Poitevin A, Van Ende A, Cardoso MV, Van Landuyt KL, *et al*. Meta-analytical review of parameters involved in dentin bonding. J Dent Res 2012; 91: 351-357.
21) De Munck J, Van Landuyt K, Peumans M, Poitevin A,

Lambrechts P, Braem M, *et al*. A critical review of the durability of adhesion to tooth tissue: methods and results. J Dent Res 2005; 84: 118-132.
22) Armstrong SR, Jessop JL, Vargas MA, Zou Y, Qian F, Campbell JA, *et al*. Effects of exogenous collagenase and cholesterol esterase on the durability of the resin-dentin bond. J Adhes Dent 2006; 8: 151-160.
23) Pashley DH. Dentin: A dynamic substrate —a review. Scanning Microsc 1989; 3: 161-74; discussion 74-76.
24) Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM, *et al*. Collagen degradation by host-derived enzymes during aging. J Dent Res 2004; 83: 216-221.
25) Brackett MG, Li N, Brackett WW, Sword RJ, Qi YP, Niu LN, *et al*. The critical barrier to progress in dentine bonding with the etch-and-rinse technique. J Dent 2011; 39: 238-248.
26) Pashley DH, Tay FR. Aggressiveness of contemporary self-etching adhesives. Part II: etching effects on unground enamel. Dent Mater 2001; 17: 430-444.
27) Awad MM, Alhalabi F, Alshehri A, Aljeaidi Z, Alrahlah A, Ozcan M, *et al*. Effect of non-thermal atmospheric plasma on micro-tensile bond strength at adhesive/dentin interface: A systematic review. Materials (Basel) 2021; 14: 1026.
28) Van Landuyt KL, Peumans M, De Munck J, Cardoso MV, Ermis B, Van Meerbeek B. Three-year clinical performance of a HEMA-free one-step self-etch adhesive in non-carious cervical lesions. Eur J Oral Sci 2011; 119: 511-516.
29) Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt KL. State of the art of self-etch adhesives. Dent Mater 2011; 27: 17-28.
30) Yoshihara K, Yoshida Y, Nagaoka N, Fukegawa D, Hayakawa S, Mine A, *et al*. Nano-controlled molecular interaction at adhesive interfaces for hard tissue reconstruction. Acta Biomater 2010; 6: 3573-3582.
31) Van Landuyt KL, Mine A, De Munck J, Countinho E, Peumans M, Jaecques S, *et al*. Technique sensitivity of water-free one-step adhesives. Dent Mater 2008; 24: 1258-1267.
32) Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, *et al*. Buonocore memorial lecture. Adhesion to enamel and dentin: Current status and future challenges. Oper Dent 2003; 28: 215-235.
33) Hirokane E, Takamizawa T, Kasahara Y, Ishii R, Tsujimoto A, Barkmeier WW, *et al*. Effect of double-layer application on the early enamel bond stregnth of universal adhesives. Clin Oral Investg 2021; 25: 907-921.
34) Hashimoto M. A review —micromorphological evidence of degradation in resin-dentin bonds and potential preventional solutions. J Biomed Mater Res B Appl Biomater 2010; 92: 268-280.
35) Feitosa VP, Sauro S, Zenobi W, Silva JC, Abuna G, Van Meerbeek B, *et al*. Degradation of adhesive-dentin interfaces created using different bonding strategies after five-year simulated pulpal pressure. J Adhes Dent 2019; 21: 199-207.
36) Inoue S, Koshiro K, Yoshida Y, De Munck J, Nagakane K, Suzuki K, *et al*. Hydrolytic stability of self-etch adhesives bonded to dentin. J Dent Res 2005; 84: 1160-1164.
37) Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, De Stefano Dorigo E. Dental adhesion review: aging and stability of the bonded interface. Dent Mater 2008; 24: 90-101.
38) Yoshihara K, Nagaoka N, Hayakawa S, Okihara T, Yoshida Y, Van Meerbeek B. Chemical interaction of glycero-phosphate dimethacrylate (GPDM) with hydroxyapatite and dentin. Dent Mater 2018; 34: 1072-1081.
39) Yoshihara K, Nagaoka N, Okihara T, Kuroboshi M, Hayakawa S, Maruo Y, *et al*. Functional monomer impurity affects adhesive performance. Dent Mater 2015; 31: 1493-1501.
40) Van Landuyt KL, Yoshida Y, Hirata I, Snauwaert J, De Munck J, Okazaki M, *et al*. Influence of the chemical structure of functional monomers on their adhesive performance. J Dent Res 2008; 87: 757-761.
41) Van Landuyt KL, Snauwaert J, Peumans M, De Munck J, Lambrechts P, Van Meerbeek B. The role of HEMA in one-step self-etch adhesives. Dent Mater 2008; 24: 1412-1419.
42) Pashley DH, Tay FR, Breschi L, Tjäderhane L, Carvalho RM, Carrilho M, *et al*. State of the art etch-and-rinse adhesives. Dent Mater 2011; 27: 1-16.
43) Chen C, Niu LN, Xie H, Zhang ZY, Zhou LQ, Jiao K, *et al*. Bonding of universal adhesives to dentine —Old wine in new bottles? J Dent 2015; 43: 525-536.
44) Hanabusa M, Mine A, Kuboki T, Momoi Y, Van Ende A, Van Meerbeek B, *et al*. Bonding effectiveness of a new 'multi-mode' adhesive to enamel and dentine. J Dent 2012; 40: 475-484.
45) Malacarne J, Carvalho RM, de Goes MF, Svizero N, Pashley DH, Tay FR, *et al*. Water sorption/solubility of dental adhesive resins. Dent Mater 2006; 22: 973-980.
46) Perdigão J, Swift EJ. Universal adhesives. J Esthet Restor Dent 2015; 27: 331-334.
47) Marchesi G, Frassetto A, Mazzoni A, Apolonio F, Diolosà M, Cadenaro M, *et al*. Adhesive performance of a multi-mode adhesive system: 1-year in vitro study. J Dent 2014; 42: 603-612.
48) Armstrong S, Breschi L, Özcan M, Pfefferkorn F, Ferrari M, Van Meerbeek B. Academy of Dental Materials guidance on in vitro testing of dental composite bonding effectiveness to dentin/enamel using micro-tensile bond strength (μTBS) approach. Dent Mater 2017; 33: 133-143.
49) International Organization for Standardization. ISO-4049:2019. Dentistry polymer-based restorative materials. Geneva: International Organization for Standardization; 2019. Available at https://www.iso.org/obp/ui/#iso:std:iso:4049:ed-5:v1:en
50) Moreira DM, Almeida JF, Ferraz CC, Gomes BP, Line SR, Zaia AA. Structural analysis of bovine root dentin after use of different endodontics auxiliary chemical substances. J Endod 2009; 35: 1023-1027.
51) Yoshida Y, Yoshihara K, Hayakawa S, Nagaoka N, Okihara T, Matsumoto T, *et al*. HEMA inhibits interfacial nano-layering of the functional monomer MDP. J Dent Res 2012; 91: 1060-1065.