Bond strength of universal adhesive applied to dry and wet dentin: one-year in vitro evaluation

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Aim: This study evaluated the influence of dentin wettability on the immediate and extended microtensile bond strength (µTBS) of a universal adhesive system used in the etch-and-rinse strategy. Methods: Twenty human third molars were selected and divided into four groups according to the adhesive system and dentin wettability. The µTBS values of each group were registered 24 h and one year after adhesive system application and resin composite block build-up (n=30). Data were analyzed by the t-test (p<0.05). Results: When both adhesive systems were compared, there was no statistically significant difference when they were applied following wet bonding (p>0.05). However, the dry bonding reduced µTBS values of the Adper Single Bond 2 adhesive (p<0.05). Regarding storage time, both groups presented similar µTBS values at 24 h and one year (p>0.05). Conclusions: Therefore, the Scotchbond Universal Adhesive can be applied to dry or wet dentin without compromising the etch-and-rinse bonding quality and the durability of the restorations. Keywords: Dental cements. Dentin. Tensile strength.
Introduction

Due to the high patient demand for esthetic restorations and minimally invasive treatments, adhesive systems have become a fundamental material for achieving the stable and long-term bonding effectiveness of aesthetic restorations to mineralized tooth tissues\(^1\). Adhesion of restorative materials to hard tissues (enamel and dentin) is challenging due to the differences in these tissues\(^2\). Dental enamel presents a structure more homogenous than that of dentin, resulting in reliable and long-term durable adhesive bonds between the enamel and the restorative material. In contrast, dentin substrate is characterized by a wide variety of inorganic and organic components; consequently, adhesive bonding to dentin is more sensitive\(^2\).

In the past, the adhesive systems available on the market have been classified into two categories: etch-and-rinse (ER) and self-etch strategies (SE)\(^3,4\). In the ER strategy, a phosphoric acid gel is first applied to the dentin substrate, followed by application of the primer and the bond resin separately or in a single solution\(^3,4\). A major disadvantage of this strategy is susceptibility to variations in the degree of dentin moisture, which is subjective and depends on operator skills\(^5\). Excessive residual moisture may hinder the impregnation of monomers into demineralized substrate by dilution of these components\(^6\). Conversely, the overdrying of the dentin surface may promote collagen fibril collapse and, consequently, the incomplete impregnation of resin monomers into the collagen fibers, decreasing the bond strength\(^7\). In addition, the dentin etching with phosphoric acid can lead to collagen matrix degradation in the dentin as a result of the activation of endogenous dentin collagenolytic enzymes by acidity\(^8,9\). This process can result in impaired bond integrity of the adhesive interface\(^8,9\).

To overcome the limitations of the ER strategy, SE adhesives were developed, characterized by acidic functional monomers that simultaneously etch and prime the tooth substrate for bonding\(^10\). Previous studies have demonstrated that the laboratory and clinical performance of traditional SE adhesives has not been satisfactory, mainly in the enamel\(^11,12\). Selective enamel etching has been suggested to improve the adhesive bond to enamel\(^13,14\). However, the application of phosphoric acid to enamel may inadvertently etch dentin, decreasing the bond strength of SE adhesives to this tissue\(^15\).

The latest trends in adhesive systems are multi-mode or universal one-bottle adhesives that may be used as either ER or SE adhesives or as SE adhesives on dentin and ER adhesives on enamel (selective enamel-etching)\(^16-18\). Most of the multi-mode or universal adhesives are characterized by 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP), which bonds ionically to dentin, forming hydrolytically stable calcium salts on hydroxyapatite in the form of "nano-layering"\(^16\). The manufacturers claim that multi-mode adhesives may also be applied to dentin under different bonding strategies (dry or wet bonding)\(^15,19\). However, there are few studies of the bonding performance and reliability of those adhesives over the short or long term when applied to dry or wet dentin\(^15,19,20\). Thus, more information is necessary to predict the long-term bonding durability of universal adhesives.

Therefore, the aim of this in vitro study was to evaluate whether the bond strength of a universal adhesive to dentin is affected by application mode (dry- and wet-bonding),...
When the etch-and-rinse strategy is used, after 24 h and one year of water storage. Three null hypotheses were set: 1) Bond strength to dentin would not be affected by application mode (dry- and wet-bonding) when the etch-and-rinse protocol is used; 2) bond strength to dentin would not be affected by storage period; and 3) there would be no difference in bond strength to dentin between etch-and-rinse and universal adhesives.

Materials and methods

Tooth selection and preparation

Twenty healthy human third molars were used in this study, after approval from the Research Ethics Committee of the University of Paraíba (protocol n. 17665613.2.0000.5188). The teeth were cleaned, stored in 0.2% thymol solution, and used within one month after extraction. All tooth roots were embedded in self-curing acrylic resin. Then, the occlusal enamel was removed by means of a diamond disc (Extec, Enfield, CT, USA) under water-cooling. The exposed occlusal dentin surfaces were wet-abraded with silicon carbide paper (600 grit) under water-cooling for 60 s by means of a polishing machine (Politriz ERIOS – 27000, São Paulo, SP, Brasil) to standardize the smear layer.

Experimental design

The teeth were randomly assigned among four groups according to the different bonding strategies of the selected adhesive systems (n = 5). The two-step etch-and-rinse adhesive, Adper Single Bond 2 (AS) (3M ESPE, St. Paul, MN, USA), and the universal adhesive, Scotchbond Universal Adhesive (SU) (3M ESPE, St. Paul, MN, USA), were applied to dentin surfaces following a dry- or a wet-bonding etch-and-rinse adhesive protocol. Composition, batch number of each material, and adhesive strategies are shown in Table 1.

Table 1. Brand, batch number, composition, and adhesive strategies of materials used.

| Adhesive system   | Manufacturer/batch number | Type                          | Composition                                                                 | Etch-and-rinse strategy                                                                 |
|-------------------|---------------------------|-------------------------------|-----------------------------------------------------------------------------|----------------------------------------------------------------------------------------|
| Scotchbond Universal | 3M/ESPE, St. Paul, MN, USA (526247) | Universal adhesive system | 10-MDP, phosphate monomer, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane | I. Apply etchant for 15 s.  
II. Rinse for 10 s.  
III. Air-dry to remove excess water.  
IV. Keep dentin moist (wet-bonding approach) or keep dentin dry. Do not overdry (dry-bonding approach).  
V. Apply 2 consecutive coats of adhesive.  
VI. Gently air-dry for 5 s. 
VII. Light-polymerize for 10 s. |
| Adper Single Bond 2 | 3M/ESPE, St. Paul, MN, USA (N49344) | Etch-and-rinse adhesive system | 1. Etchant: 35% phosphoric acid (Scotchbond Etchant).  
2. Adhesive: bis-GMA, HEMA, dimethacrylates, ethanol, water, photoinitiator, methacrylate functional copolymer of polyacrylic and poly(itaconic) acids, 10% by weight of 5-nm-diameter spherical silica particles | I. Apply etchant for 15 s.  
II. Rinse for 10 s.  
III. Air-dry to remove excess water.  
IV. Keep dentin moist (wet-bonding approach) or keep dentin dry. Do not overdry (dry-bonding approach).  
V. Apply 2 consecutive coats of adhesive.  
VI. Gently air-dry for 5 s. 
VII. Light-polymerize for 10 s. |
Restorative procedure and specimen preparation

After the bonding process, three resin composite increments (Z100-3M ESPE, St. Paul, MN, USA) of 1.5 mm were placed on the dentin surface, and each increment was light-cured for 40 s by means of a LED light-curing unit set at 400 mW/cm² (GNATUS, São Paulo, SP, Brazil). The restored teeth were then stored in distilled water at 37°C (± 1°C) for 24 h.

After this storage period, the specimens were sectioned longitudinally in the mesio-distal and buccal-lingual directions across the bonded interface, by means of a slow-speed diamond disc (Labcut 1010, Extec, Enfield, CT, USA). Then, the specimens were sectioned transversely in the cervical region to obtain bars measuring 1 mm² x 10 mm². Half of the bars obtained from each tooth were used immediately (24 h) for the micro-tensile bond strength (μTBS) test, while the other half were stored in distilled water at 37°C (± 1°C) for one year and then subjected to μTBS testing. For μTBS testing, the bars were fixed to a testing jig with cyanoacrylate glue (Super Bonder Gel – Loctite Brasil Ltda) and subjected to tensile load at a crosshead speed of 0.5 mm/min until failure (microtensor OM-100 machine, Odeme, Luzerna, SC, Brasil). The μTBS values (MPa) were calculated by dividing the load at failure by the cross-sectional bonding area. The fractured surfaces of all specimens were observed by means of an optical microscope (XJM-400, KOZO, Nanjing, China) at a magnification of 100x, and fracture patterns were classified as (1) cohesive failure in adhesive, (2) cohesive failure in dentin, (3) cohesive failure in the hybrid layer, or (4) mixed failure (cohesive failure in adhesive and cohesive failure in the hybrid layer). The data from fracture patterns were analyzed by descriptive statistics.

The experimental unit in the current study was the bar. An average of 10 to 15 bars was obtained from each tooth, with the experimental group (n = 30) having the smallest number of test specimens, the experimental groups are in the figure 1. Thus, the μTBS value of each bar was used for statistical analysis. The data from μTBS were analyzed by the t-test for independent samples (α = 0.05). In addition, the reliability of the bond

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**Figure 1. Sample diagram.**
strength for each group was analyzed by Weibull analysis. The Weibull moduli (shape parameter) (slope of the line relating applied stress and the probability of specimen failure, m) were calculated, applying maximum likelihood estimation. The 95% upper and lower confidence intervals were calculated using the likelihood ratio (MINITAB 17.0, State College, Pennsylvania, USA). Differences between the paired values for m were considered significant when the 95% confidence intervals did not overlap.

Results

The bond strength means (MPa) and standard deviations for all experimental groups are presented in Table 2.

Table 2. Microtensile bond strength (MPa) values of adhesive systems to dentin among the test groups, comparing materials and different etch-and-rinse strategies.

| Storage time | Etch-and-rinse strategy | Adhesive system          |
|--------------|-------------------------|--------------------------|
|              |                         | Scotchbond Universal (SU)| Adper Single Bond 2 (AS)|
| 24 hours     | wet-bonding             | 49.08 (15.23)$^{Aa}$      | 47.38 (23.23)$^{Aa}$     |
|              | dry-bonding             | 52.28 (19.43)$^{Aa}$      | 26.91 (10.25)$^{Bb}$     |
| One year     | wet-bonding             | 50.22 (16.36)$^{Aa}$      | 45.13 (18.61)$^{Aa}$     |
|              | dry-bonding             | 53.87 (23.51)$^{Aa}$      | 21.63 (13.91)$^{Bb}$     |

Means followed by different uppercase letters in the same row indicate statistically significant differences between adhesive systems within the same etch-and-rinse strategy and evaluation time. Means followed by different lowercase letters on the same column indicate statistically significant differences between etch-and-rinse strategy within the same adhesive system and evaluation time ($p < 0.05$).

The SU adhesive showed no difference in µTBS values between dry- and wet-bonding etch-and-rinse strategies in 24 hours and after one year ($p=0.48$). Conversely, the AS adhesive applied to a dry dentin surface presented significantly lower µTBS values than that measured when the adhesive was applied to the dentin following a wet-bonding technique ($p<0.001$). When both adhesive systems were compared, there was no statistically significant difference between them when they were applied following a wet-bonding strategy in 24 hours ($p=0.74$) and after one year ($p=0.26$). However, when the adhesive systems were applied to dry dentin, the SU showed significantly higher µTBS values ($p<0.001$) for both storage times.

Regarding storage time, both adhesive systems presented similar µTBS values at 24 hours and one year ($p>0.05$), regardless of the etch-and-rinse strategy (dry or wet dentin).

The Weibull analysis indicated no change in the Weibull parameter for SU adhesive for all study conditions. On the other hand, AS adhesive showed the lowest m values applied to the dentin following a wet-bonding (24 hours) and dry-bonding (one year) technique. Comparing the adhesive systems, SU demonstrated higher m values than
AS for wet-bonding strategy in 24 hours and dry-bonding strategy in one year. This finding suggests a more predictable, consistent performance of SU product than seen when using the AS adhesive (Table 3, Figure 2, and 3).

Table 3. Weibull moduli ($m$) values, among the experimental groups comparing different adhesive system, storage time, and etch-and-rinse strategy.

| Storage time | Etch-and-rinse strategy | Scotchbond Universal (SU) | Adper Single Bond 2 (AS) |
|--------------|--------------------------|---------------------------|--------------------------|
|              |                          | 3.6 (2.73-4.74)$^{AA}$    | 2.23 (1.67-2.98)$^{AB}$  |
| 24 hours     | wet-bonding              |                           |                          |
|              | dry-bonding              | 3.12 (2.32-4.2)$^{AA}$    | 2.80 (2.16-3.63)$^{AB}$  |
| One year     | wet-bonding              | 3.37 (2.57-4.42)$^{AA}$    | 2.68 (2.02-3.56)$^{AA}$  |
|              | dry-bonding              | 2.57 (1.9-3.46)$^{BB}$    | 1.68 (1.28-2.19)$^{BB}$  |

Means followed by different uppercase letters in the same row indicate statistically significant differences between adhesive systems within the same etch-and-rinse strategy and evaluation time. Means followed by different lowercase letters on the same column indicate statistically significant differences between etch-and-rinse strategy within the same adhesive system and evaluation time ($p < 0.05$).

Figure 2. Weibull distribution plots of microtensile bond strength data for different etch-and-rinse strategy and evaluation time within the same adhesive system. ASW- Adper Single Bond 2 wet-bonding; ASD- Adper Single Bond 2 dry-bonding.

The percentages of specimens according to fracture mode for all experimental groups at 24 h and one year are summarized in Tables 4 and 5, respectively. The predominant failure patterns observed were mixed failure, cohesive failure in adhesive, and cohesive failure in the hybrid layer (IV).
Figure 3. Weibull distribution plots of microtensile bond strength data for different etch-and-rinse strategy and evaluation time for Scotchbond Universal adhesive system. SUW- Scotchbond Universal wet-bonding; SUD- Scotchbond Universal dry-bonding.

Table 4. Percentages (%) of specimens according to the fracture mode of all test groups in 24 hours’ storage time.

| Adhesive systems     | Etch-and-rinse strategy | Fracture patterns (%) |
|----------------------|-------------------------|-----------------------|
|                      |                         | I         | II        | III       | IV        |
| Scotchbond Universal | wet-bonding             | 23.3      | 0         | 3.3       | 73.4      |
|                      | dry-bonding             | 13.3      | 0         | 13.3      | 73.4      |
| Adper Single Bond 2  | wet-bonding             | 16.5      | 0         | 0         | 86.7      |
|                      | dry-bonding             | 13.3      | 0         | 20        | 66.7      |

Table 5. Percentages (%) of specimens according to the fracture mode of all test groups in one-year storage time.

| Adhesive systems     | Etch-and-rinse strategy | Fracture patterns (%) |
|----------------------|-------------------------|-----------------------|
|                      |                         | I         | II        | III       | IV        |
| Scotchbond Universal | wet-bonding             | 0         | 0         | 0         | 100       |
|                      | dry-bonding             | 20        | 0         | 0         | 80        |
| Adper Single Bond 2  | wet-bonding             | 13.3      | 0         | 0         | 86.7      |
|                      | dry-bonding             | 50        | 0         | 0         | 50        |
Discussion

Based on the results of this in vitro study, the universal adhesive etch-and-rinse strategy can be applied to dry and wet dentin surfaces, since dry- and wet-bonding strategies demonstrated suitable bond strength to dentin in short (24 h) and long periods of aging (one year). In contrast, the dry-bonding mode had a negative impact on dentin bond quality of the two-step adhesive, and, consequently, this material presented the worst bond strength values when applied to dry dentin. Thus, the null hypotheses of the present study were rejected.

The universal adhesives were introduced to simplify and optimize the adhesive procedures. These materials can be used in different types of adherent substrate and etching modes (etch-and-rinse or self-etch). A recent systematic review and meta-analysis showed revealed that the mild universal adhesives seem stable materials, in both etch-and-rinse or self-etch strategies. In this study, the universal adhesive was used in etch-and-rinse mode. As is known, the SU adhesive tested in this study contains 10-MDP monomer, which provides chemical bonding to hydroxyapatite, forming hydrolytically stable calcium salts. Additionally, this adhesive contains a polyalkenoic acid copolymer that also interacts ionically with the hydroxyapatite through the carboxylic groups. To trigger the ionization of the phosphate monomer and polyalkenoic acid copolymer, a certain amount of water (10–15% by wt) (3M ESPE) was added to the universal adhesive. Thus, the unique composition of the universal adhesive (SU) can explain the results observed in this study, which revealed no statistically significant difference for µTBS values between the dry- or wet-bonding etch-and-rinse strategy after 24 hours and one year. The chemical components and the water contained in SU adhesives may be able to rehydrate collagen fibrils, allowing for the re-expansion of the interfibrillar spaces for the infiltration of resin monomers and the formation of resin tags branching out profusely into dentin tubules, permitting a satisfactory dentin seal and performance of those adhesives, even with dry-demineralized dentin.

The results of this study are in agreement with those of other reports, which concluded that the bonding durability of the universal adhesive was acceptable and did not seem to vary depending on the etching and application mode (dry- or wet-bonding). This outcome was confirmed by Weibull analysis that revealed how reliable a given treatment is. The high m values observed for SU adhesive (table 3, figure 1), mainly with dry-demineralized dentin, suggest that the universal adhesive investigated can be considered reliable over time.

Conversely, µTBS values for the conventional two-step etch-and-rinse adhesive (AS) decreased when this adhesive was applied to a dry-dentin surface in the etch-and-rinse protocol after 24 h and one year. This can be attributed to an insufficient amount of water within the dentin structure, which can lead to a collapsed collagen network. Consequently, reduced resin monomer penetration into the entire depth of the demineralized dentin can occur when the AS adhesive is used as the etch-and-rinse adhesive to dry dentin, corroborating with other studies. The low m values observed for the AS adhesive with dry dentin (table 3, figure 2) ratify that the application of this material to wet dentin is recommended to obtain mechanical stability of the bond to dentin.
Regarding failure patterns, mixed failure (cohesive failure in adhesive and cohesive failure in the hybrid layer) was the predominant mode for all experimental groups. This finding is in agreement with other studies\textsuperscript{5,33-36} and suggests that the bonding agents interacted with dental substrates during monomer infiltration by forming a hybrid layer. However, this layer fractured due to concentrated tension at the adhesive interface.

One of the main degradation mechanisms of dentin bonding is the hydrolysis of the collagen fibrils and the polymerized resin matrix in the adhesive layer\textsuperscript{37}. However, the patterns of degradation of resin-dentin bonding depend on the type of adhesive system\textsuperscript{37}. In this study, $\mu$TBS values for both adhesives tested did not decrease significantly after one year of storage in distilled water. Therefore, the storage medium used in this research did not influence the long-term stability of the bond-adhesive interface.

Therefore, according to the present study, the SU adhesive may be an effective alternative approach for dentin restorations. Nevertheless, other factors related to the oral environment may have a more complex influence on the bonding performance of adhesives. Thus, future investigations should be conducted for the clinical evaluation of the SU adhesive to confirm the results presented in this research.

In conclusion, the universal adhesive tested in this study can be applied to dry or wet demineralized dentin without compromising the etch-and-rinse bonding quality and the durability of the restorations. On the other hand, the dry-bonding etch-and-rinse adhesive protocol influenced negatively the dentin bond quality of the conventional two-step adhesive.

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