Introduction

The clinical success of resin composite restorations depends on the effectiveness and durability of the adhesive interface (1). In contrast to enamel, bonding to dentin remains challenging because of the composition of the dentin, which has lower inorganic content, higher amount of water, and presence of collagen fibrils, in addition to other histological features such as morphological differences in dentin regions (2). In addition, the permeability of dentin increases significantly with cavity depth due to differences in the shape and number of tubules between the superficial and deeper dentin areas near the pulp (3).

Current dental adhesive systems can be classified according to the strategy that they bond to dental tissues in etch-and-rinse adhesives, which require prior etching of surfaces with 37% phosphoric acid, or self-etch adhesives, which do not require prior acid etching and bond to dental tissues by means of acidic primers. When adequately performed, these two bonding strategies may demonstrate good results in long-term bonding (4). However, there is clinical evidence of a better performance of resin composite restorations bonded to dentin when two-step, self-etch adhesives with intermediate strong aggressiveness (i.e., pH>1 and <2) are used as compared with two-step, etch-and-rinse adhesives (5). Nonetheless, etch-and-rinse adhesives are still prevalent in many international dental markets.

As a commercial strategy from dental product companies and potentially with the aim of simplifying the bonding procedures, adhesive systems denominated “universal” or “multimode” were introduced. These adhesives are supposedly designed to promote adhesion to various restorative substrates, e.g. enamel, dentin, resin composites, ceramics, and metal alloys, although indications among products vary. Most universal adhesives also can be applied to the dentin as two-step, etch-and-rinse or one-step, self-etch bonding agents (3,6,7). Laboratory studies (8,9) and clinical trials (10,11) have shown that universal adhesives seem to have a similar performance in bonding to dentin as compared with two-step, etch-and-rinse systems. However, although several commercial brands are available in the market, most of the available evidence in the literature reports findings from a single universal adhesive (i.e., Single Bond Universal, 3M ESPE, St. Paul, MN, USA), which was one of the first to mention its “universal” character. There is room for evaluating other multi-mode adhesives, especially applied to the dentin using different bonding strategies.
The aim of the present study was to evaluate the bond strength (immediate and after 6 months of aging) of universal adhesives applied to dentin using etch-and-rinse or self-etch bonding strategies. Gold standard adhesives were tested as references for each adhesion strategy. The null-hypothesis was that the universal adhesive systems would perform similarly to each other, irrespective of the bonding strategy to dentin.

**Material and Methods**

**Study Design and Tested Materials**

In this in vitro study, a 6×2×2 factorial design was used to investigate the effect of the following factors on bond strength of resin composite to dentin: adhesive system (Ambar Universal, G-Bond, Single Bond Universal, Tetric N-Bond Universal, Ybond Universal, or control), bonding strategy (etch-and-rinse or self-etch modes), and aging time (24 h or 6 months of storage in water at 37°C). The manufacturer of G-Bond does not use the term “universal” but indicates that the adhesive is “effective in all conditions” of dentin. A three-step, etch-and-rinse adhesive system (Scotchbond Multipurpose Plus) and a two-step, self-etch adhesive system (Clearfil SE Bond) were tested in the control groups for each bonding strategy. The compositions and manufacturer information of the adhesives tested are presented in Table 1. The primary response-variable was microtensile bond strength to dentin. In each group, an average of 35 beam-shaped (stick) composite-dentin bonded specimens originated from 5 teeth were tested (the tooth was the experimental unit). Failure modes of the specimens fractured in the bond strength test were classified under magnification. The pH of the adhesives (n=3) was measured using a digital pH meter (HI2221 Calibration Check/ORP Meter; Hanna instruments, Woonsocket, RI, USA). Degree of C=C conversion (DC, n=3) of the adhesives was evaluated using Fourier-transform infrared spectroscopy. For Clearfil SE Bond and Scotchbond Multipurpose Plus, the pH of the primer was measured.

**Table 1. Compositions of the adhesive systems tested**

| Adhesive System                  | Composition                                                                                      |
|---------------------------------|-------------------------------------------------------------------------------------------------|
| Ambar Universal                 | FGM, Joinville, SC, Brazil 070116 UDMA, HEMA, methacrylate hydrophilic monomers, methacrylate acid monomers, ethanol, water, silanized silicon dioxide, camphorquinone, ethyl 4-dimethylamino-benzoate, surfactant, sodium fluoride |
| G-Bond                          | GC, Tokyo, Japan 1309181 Acetone, UDMA, dimethacrylate component, phosphoric acid ester monomer, photocatalyst |
| Single Bond Universal           | 3M ESPE, St. Paul, MN, USA 619545 MDP phosphate monomer, dimethacrylate resins, HEMA, polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane |
| Tetric N-Bond Universal         | Ivoclar Schaan, Liechtenstein U42905 Ethanol, phosphonic acid acrylate, Bis-GMA, HEMA, UDMA, diphenyl (2,4,6- trimethylbenzoyl) phosphine oxide |
| Ybond Universal                 | Yller, Pelotas, RS, Brazil 2045 Hydrophilic methacrylate monomers, hydrophobic methacrylate monomers, initiators, stabilizers, silane, ethanol, water, silanized nanoparticles |
| Clearfil SE Bond                | Kuraray, Osaka, Japan 9N0168 9U0270 Primer: MDP, dimethacrylate monomer, HEMA, silica, N,N-diethanol-p-toluïdine, CQ; Bond: HEMA, dimethacrylate monomer, Bis-GMA, N,N-diethanol-p-toluïdine, silica, camphorquinone |
| Scotchbond Multipurpose Plus    | 3M ESPE N782259 N763504 Primer: Polyalkenoic acid copolymer, HEMA, water, ethanol; Bond: Bis-GMA, HEMA, tertiary amines, photoinitiator |

*Data obtained from the manufacturers.
dentin and the dentin surfaces were wet-polished using 600-grit SiC abrasive papers. The teeth were randomly divided into 24 groups (n=5 per group), according to the adhesive system, bonding technique, and storage time tested. The adhesive systems were applied at room conditions according to the manufacturers’ recommendations, as detailed in Table 2. The specimens were prepared in a randomized order. Resin composite restorations were built up on the dentin surfaces using three increments of resin composite shade A3E (Filtek Z350; 3M ESPE). Each increment was light-activated for 20 s. Preparation of the specimens was carried out randomly. After 24 h storage in distilled water, the dentin-resin composite restorations were cut into beam-shaped specimens with approximately 1 mm² bonded area using a diamond saw under running water (Isomet 1000; Lake Bluff, IL, USA). Each tooth yielded an average of 14 sticks and all specimens were tested.

From each restored tooth, half the number of beam-shaped composite-dentin bonded specimens were immediately tested (24 h) and the other half was stored in distilled water at 37°C for 6 months. The stick specimens were randomly assigned to the aging times and stored in Eppendorf tubes placed in an oven. The storage medium was renewed every month. The microtensile bond strength tests were carried out on a mechanical testing machine (DL500; São José dos Pinhais, PR, Brazil). The specimens were fixed to a notched active gripping device and tested under tensile stress at a crosshead speed of 1 mm/min until failure. Bond strength values (MPa) for all beam-shaped specimens tested from a same tooth assigned to each

Table 2. Details of the application of the adhesive systems to dentin following the manufacturers’ directions

| Adhesive        | Bonding strategy | Application steps                                                                                                                                 |
|-----------------|------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|
| Ambar Universal | Etch-and-rinse   | The dentin was acid-etched with 37% phosphoric acid for 15 s, washed with air/water spray for 15 s, excess dentin moisture water was removed with absorbent paper, a first layer of adhesive was applied and rubbed for 10 s, the solvent was evaporated with air stream for 10 s, the adhesive was photoactivated for 10 s |
| Self-etch       |                  | The surface was washed with air/water spray, excess dentin moisture water was removed with absorbent paper, a first layer of adhesive was applied and rubbed for 10 s, then a second adhesive layer was applied, the solvent was evaporated with air stream for 10 s, the adhesive was photoactivated for 10 s |
| Etch-and-rinse  |                  | The dentin was acid-etched with 37% phosphoric acid for 15 s, washed with air/water spray for 15 s, excess dentin moisture water was removed with absorbent paper, the adhesive was actively applied for 10 s followed by solvent evaporation with air stream for 10 s, the adhesive was photoactivated for 20 s |
| G-Bond          | Self-etch        | The surface was washed with air/water spray, excess dentin moisture water was removed with absorbent paper, the adhesive was applied and rubbed for 10 s, the solvent was evaporated with air stream for 5 s, the adhesive was photoactivated for 20 s |
| Single Bond     | Etch-and-rinse   | The dentin was acid-etched with 37% phosphoric acid for 15 s, washed with air/water spray for 15 s, excess dentin moisture water was removed with absorbent paper, the adhesive was actively applied for 20 s, the solvent was evaporated with air stream for 5 s, the adhesive was photoactivated for 10 s |
| Universal       | Self-etch        | The surface was washed with air/water spray, excess dentin moisture water was removed with absorbent paper, the adhesive was applied and rubbed for 20 s, the solvent was evaporated with air stream for 5 s, the adhesive was photoactivated for 10 s |
| Tetric N-Bond   | Etch-and-rinse   | The dentin was acid-etched with 37% phosphoric acid for 15 s, washed with air/water spray for 15 s, excess dentin moisture water was removed with absorbent paper, the adhesive was actively applied for 20 s, the solvent was evaporated with air stream for 5 s, the adhesive was photoactivated for 10 s |
| Universal       | Self-etch        | The surface was washed with air/water spray, excess dentin moisture water was removed with absorbent paper, the adhesive was applied and rubbed for 20 s, the solvent was evaporated with air stream for 5 s, the adhesive was photoactivated for 10 s |
| Scotchbond      | Etch-and-rinse   | The surface was washed with air/water spray, excess dentin moisture water was removed with absorbent paper, two layers of adhesive were applied and each rubbed for 20 s, the solvent was evaporated with air stream for 5 s, the adhesive was photoactivated for 20 s |
| Multipurpose Plus | Self-etch       | The surface was washed with air/water spray, excess dentin moisture water was removed with absorbent paper, the primer was applied and gently air-dried with air stream for 5 s, the adhesive was applied and photoactivated for 10 s |
| Clearfil SE Bond | Self-etch       | The surface was washed with air/water spray, excess dentin moisture water was removed with absorbent paper, the primer was applied and rubbed for 20 s, the solvent was evaporated with air stream for 5 s, the adhesive was applied and photoactivated for 20 s |
aging time were averaged, thus the tooth was considered the experimental unit (n=5 per group). After the tests, all surfaces were examined using a light stereomicroscope at 40x magnification to classify the failure modes: adhesive (interfacial) failure, cohesive failure in dentin, cohesive failure in resin composite, or mixed failure (involving more than one failure mode). The predominance of each failure type at the surface was considered in the classification. Pre-testing failures (premature debondings) were also registered. Specimens with failures classified as premature or cohesive were not considered in the calculation of the microtensile bond strength values. For each group, at least five fractured specimens were coated with carbon and examined using scanning electron microscopy (JSM 6610; JEOL, Tokyo, Japan).

**Statistical Analysis**

Data for pH and DC were analyzed using One-Way Analysis of Variance (ANOVA). Bond strength data were submitted to a Three-Way ANOVA (adhesive system×bonding strategy×aging time). All pairwise multiple comparison procedures were carried out using the Student-Newman–Keuls’ post hoc method. The statistical tests were performed at α=0.05.

**Results**

**Ph and C= C Conversion**

Table 3 shows the results for the pH and DC. All adhesives showed significant differences in pH when compared with each other (p<0.001). The primer of Scotchbond was the least acidic material and Ybond Universal the most acidic adhesive. According to the aggressiveness of self-etch adhesives, Ybond Universal and the primer of Clearfil SE Bond can be classified as intermediately strong, whereas all the other universal adhesives tested can be classified as having ultra-mild aggressiveness (pH≥2.5). The DC also was significantly different among almost all adhesives tested, except between Ambar Universal and Clearfil SE Bond, and between Ybond Universal and Tetric-N-Bond Universal or Scotchbond.

The highest DC was observed for G-Bond, while Single Bond Universal had the lowest DC.

**Dentin Bond Strength**

Results for the 24 h and 6-month dentin bond strengths are shown in Table 4. One should note that only adhesive and mixed failures were accounted when calculating the bond strength values. The statistical analysis showed that the factors adhesive system and aging time as well as their interaction were statistically significant (p<0.001). The factor bonding strategy (p=0.758) and all other interactions (p≥0.207) were not significant. In the etch and rinse strategy at 24 h, all adhesives showed similar results except for G-Bond, which had significantly lower bond strength than most adhesives. After 6 months, Ybond...
Universal, Single Bond Universal, and Ambar Universal had significantly higher bond strength than Tetric-N-Bond Universal, whereas G-Bond showed significantly lower bond strength than all other materials. G-Bond and Tetric-N-Bond Universal showed significantly lower bond strengths after 6 months compared with 24 h, whereas the other adhesives had stable dentin bonding abilities in the etch-and-rinse strategy. The highest drops in bond strengths after aging were observed for G-Bond and Tetric-N-Bond Universal, whereas Ybond Universal had no bond strength drop during aging. In the self-etch strategy at 24 h, G-Bond had the lowest bond strength compared to all adhesives except Ybond. After 6 months, Ambar Universal was the only adhesive showing significantly lower dentin bond strength compared with 24 h, also having the highest drop in average bond strength during aging. Most adhesives had discreet drops in dentin bond strengths during aging when used in the self-etch strategy, with results usually comparable to the control groups.

**Failure Modes**

Results for the failure mode analysis for all beam-shaped specimens tested in each group are shown in Figure 1. Scotchbond showed a predominance of cohesive failures in both aging times, whereas by comparison Clearfil SE Bond had more adhesive and mixed failures. These two gold standard adhesives showed increased adhesive and pre-testing failures after 6 months as compared with 24 h. Ybond Universal showed more adhesive failures in the self-etch than in the etch-and-rinse mode. After storage, adhesive failures were even more frequent in either bonding strategies. Pre-testing failures for Ybond Universal at 24 h were observed only for the self-etch mode, whereas after 6 months, pre-testing failures were registered for both bonding strategies.

Cohesive failures were predominant for Single Bond Universal tested in either bonding strategies. For the etch-and-rinse mode, the frequency of adhesive and premature failures increased after storage. Tetric-N-Bond Universal had pre-testing failures only when applied using the self-etch strategy. The frequency of adhesive failures increased after storage, especially in the etch-and-rinse mode. G-Bond had most failures classified as adhesive in all testing conditions except in the etch-and-rinse.
mode after 6 months, when the frequency of pre-testing failures was substantially increased. Ambar Universal also showed increased frequency of pre-testing failures after storage, particularly when used in the self-etch mode. SEM pictures of each failure mode are shown in Figure 2. The failure modes observed were very typical of dentin bond

Figure 2. SEM pictures of specimens fractured in the bond strength analysis. Left hand side: x90 magnification; right hand side: x500 magnification. Adhesive failures generally involved the adhesive layer at the interface, whereas mixed failures in general involved a portion of dentin and/or composite. In the higher magnification, porosity can be seen within the resin-based materials. In cohesive failures, only dentin or resin composite can be observed at the failure sites.
strength tests with cracks origins observed at the corners of the specimens. Adhesive failures generally involved the adhesive layer at the interface, whereas mixed failures in general involved a portion of dentin and/or composite. In the higher magnification (Fig. 2), porosity can be seen within the resin-based materials. In cohesive failures, only dentin or resin composite can be observed at the failure sites.

Discussion

The universal adhesive systems tested in this study had different performances in bonding to dentin when compared to each other. The bonding ability of the universal adhesives and failure modes were material dependent. The bonding strategy (i.e., etch-and-rinse or self-etch approach) influenced the failure modes but did not have significant effect on the bond strength of the adhesives. Finally, in some cases differences between the dentin bonding ability of the universal adhesives compared to the gold standard materials were observed. Taking all these results into account, the null-hypothesis tested could not be accepted.

The concept of having a single material able to bond adequately to the many substrates that can be found in the mouth is quite interesting. Bonding resin-based materials to different surfaces for varied applications is common in restorative dentistry. Having one specific adhesive intended for use in each bonding scenario would be not only impractical but also not cost-effective. From the standpoint of formulating multi-purpose bonding agents, it is a hard task to put many components into a single bottle (or two bottles) and expect a good bonding performance in all situations. For instance, some universal adhesives, including the Single Bond Universal tested here, have silane in their formulation, which theoretically would allow bonding to acid-sensitive ceramics without the use of a separate application of silane. However, it has been shown that the silane molecules are not stable when mixed with acidic species in universal adhesives (13) and that application of a separate silane layer to acid-etched, glass-rich ceramics should not be left out clinically (13, 14). Another study (15) tested the use of a universal adhesive for bonding to a zirconia ceramic and reported that a silica coating method followed by silanization promoted higher bond strength than application of the universal adhesive alone.

The present results indicate that the ability in bonding to dentin of the universal adhesives, considering both the adhesion strategy and aging times, was material dependent. The same effect was observed in the failure modes. This means that the formulation of the materials tested, including type and quantity of acidic monomers, hydrophobic and hydrophilic methacrylates, solvents, photoinitiators, and other components might affect their bonding performance. Many universal adhesives are available in the market nowadays. On one hand, this can be seen as positive fact because many companies are interested in developing new materials for clinical use. On the other hand, the negative aspect of the availability of several adhesives is that it might make the dentist to have a hard time in choosing an adhesive to purchase. In that scenario, perhaps the dentist will rely on the marketing strategies and information provided by the manufacturers in the decision process instead of appropriate evidence.

This is what makes this sort of analysis reported here important in the literature. A recent study showed that there is room for improving the evidence-based practice among dentists, who often report to read articles and rely on expert’s opinions for clinical decisions (16). One of the positive findings of the present study is the observation that most universal adhesives tested had bonding performances similar to the gold standard references, either before or after water storage.

A recent systematic review of in vitro studies (17) reported that the enamel bond strength of universal adhesives is improved with prior phosphoric acid etching, whereas the same effect was not evident for dentin when mild universal adhesives were used. A similar result was observed in the present study, since no overall differences in bond strength were observed between the bonding strategies. However, the occurrence of a more stable dentin bonding in the self-etch strategy is suggested by the general observation of lower average drops in bond strength after aging. The only exception was Ambar Universal, which had a more stable dentin bond when applied in the etch-and-rinse mode. These findings can be contrasted with those reported in previous studies. Two studies observed that the dentin bond strength using a universal adhesive was similar in the etch-and-rinse and self-etch strategies (6, 7), whereas another investigation found higher bond strength in the etch-and-rinse mode (18). However, three things should be pointed out when contrasting the current findings from those of these other three studies. The first is that all three studies (6, 7, 18) tested only one multi-mode adhesive, whereas five universal adhesives were tested herein. The second point is that, although the dentin bond strengths were similar in either bonding strategies in the study by Hanabusa et al. (6), the authors reported that the resultant adhesive interface in the etch-and-rinse approach appeared ultra-structurally more vulnerable to biodegradation compared with the self-etch approach. This finding corroborates those observed in the present investigation regarding the drop in bond strength after aging. The third point is that only immediate bond strengths were evaluated in those three studies (6, 7, 18) and the overall results could be different if the specimens
were submitted to any aging regimen.

In the present investigation, the specimens were stored in water at 37 °C for six months in order to age the adhesive interfaces. According to a recent guidance paper for testing dentin bond strength using microtensile tests, a 6-month storage in water can be considered a medium to long term aging period (19). In order to accelerate the degradation process, the small beam-shaped specimens were stored, not the whole tooth-composite bonded assemblies (20,21). In addition, the water in which the specimens were soaked was renewed every month to accelerate the hydrolytic process, avoiding saturation of the storage medium. Differences in bond strength between 24 h and 6 months were observed for some materials. In addition, all adhesives tested had increased frequency of adhesive and/or pre-testing failures after storage, indicating that it was actually able to age and challenge the bonded interfaces. The presence of aging is as important factor in the study. For instance, materials that present high immediate bond strength values but reduced bond strengths after aging may reflect adhesives that are not actually able to bond well to dentin. From the clinical standpoint, it is desirable that adhesives have stable dentin bonds during aging, even if they have yielded modest initial bond strength values.

Most adhesives had dentin bonds that can be considered stable within the storage time tested, particularly when the materials were applied as self-etch adhesives. However, it seems that G-Bond and Tetric-N-Bond Universal preferably should not be used as etch-and-rinse adhesives, since in that mode they showed 40% or more average drop in bond strength after aging. G-Bond also showed a high frequency of pre-testing failures upon storage when preceded by acid etching, reinforcing the evidence of an unstable bond. The use of Ambar Universal as self-etch adhesive warrants further investigation because an average drop of 37% in bond strength was observed after aging, whereas a more stable bonding performance was observed when the same adhesive was preceded by dentin acid etching. Interestingly, G-Bond and Ambar Universal showed the highest C=C conversion among all adhesives tested. The monomer conversion of the adhesives as well their pH seemed not to be associated with their resulting dentin bonding abilities. The pH is mainly affect by the presence of acidic species in the formulation (22), with potential effects in the long-term mechanical properties and stability of the bonded interface if not buffered by dentin or intrinsic material components (23). In contrast, the C=C conversion can be affected by photoinactivation conditions and many formulation characteristics of dental adhesives (24), which unfortunately are not fully detailed in commercial formulations.

In conclusion, the present study indicates that the bonding performance of universal adhesives to dentin is material dependent. Most bonding agents tested had stable dentin bonds, with results comparable to the gold standard materials tested, particularly when the universal adhesives were applied in the self-etch mode. In general, it seems that the application of universal adhesives to dentin should not be preceded by phosphoric acid etching, although a case-by-case analysis is warranted.

Resumo

Este estudo avaliou a resistência de união à dentina imediata e após 6 meses de adesivos universais utilizando estratégias de união convencional ou autocondicionante. Os adesivos testados foram Ambar Universal, G-Bond, Single Bond Universal, Tetric N-Bond Universal e Ybond Universal. Adesivos padrão-ouro (Scotchbond Multipurpose Plus e Clearfil SE Bond) foram os controles. Resistência de união à microtração na dentina (n=5 dentes), pH e conversão de C=C (n=3) foram avaliados. Os dados foram analisados considerando α=0,05. Todos os adesivos mostraram diferenças de pH comparados aos demais. Ybond Universal teve agressividade intermediária forte, enquanto os outros foram ultramoderados. A conversão de C=C foi diferente em quase todos os adesivos. No modo convencional, em geral todos os adesivos mostraram resultados similares exceto G-Bond, que teve menor resistência de união que a maioria dos adesivos. G-Bond e Tetric-N-Bond mostraram resistências de união menores após 6 meses comparados a 24 h, enquanto os outros adesivos apresentaram estável união à dentina. No modo autocondicionante, G-Bond teve menor resistência de união que quase todos os adesivos. Após 6 meses, Ambar foi o único adesivo mostrando menor resistência de união à dentina comparado a 24 h. A maioria dos adesivos teve discreta queda na resistência de união durante o envelhecimento quando usados na estratégia autocondicionante. Os modos de falha também foram material dependentes, com um padrão geral de mais falhas adesivas e/ou pré-teste após o armazenamento. Em conclusão, o desempenho de união de adesivos universais à dentina é material dependente. Grande parte dos adesivos apresentou estável união à dentina com resultados comparáveis ao materiais padrão-ouro, particularmente quando aplicados no modo autocondicionante. Em geral, parece que o uso de adesivos universais em dentina não deve ser precedido por condicionamento com ácido fosfórico.

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