Influence of a hydrophobic resin coating on the microtensile dentin bond strength of a universal adhesive: *in vitro* study

Nuno Miguel Silva Ramos

**Orientador:** Prof. Doutor João Carlos Ramos  
**Coorientadora:** Doutora Alexandra Vinagre

Coimbra, julho 2015
Influence of a hydrophobic resin coating on the microtensile dentin bond strength of a universal adhesive: *in vitro study*

Ramos N*, Vinagre A**, Ramos J C***

* 5th year student of Integrated Master in Dentistry, Faculty of Medicine, University of Coimbra

** Invited Assistant of Integrated Master in Dentistry, Faculty of Medicine, University of Coimbra

*** Assistant Professor of Integrated Master in Dentistry, Faculty of Medicine, University of Coimbra

Área de Medicina Dentária, Faculdade de Medicina, Universidade de Coimbra
Avenida Bissaya Barreto, Blocos de Celas
3000-075 Coimbra, Portugal
Tel.: +351 239 484 183
Fax: +351 239 402 910

Author’s e-mail: nuno20ramos@gmail.com
Influence of a hydrophobic resin coating on the microtensile dentin bond strength of a universal adhesive: *in vitro* study

**Contents**

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>4</td>
</tr>
<tr>
<td>Introduction</td>
<td>5</td>
</tr>
<tr>
<td>Materials and methods</td>
<td>8</td>
</tr>
<tr>
<td>Results</td>
<td>13</td>
</tr>
<tr>
<td>Discussion</td>
<td>15</td>
</tr>
<tr>
<td>Conclusion</td>
<td>21</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>22</td>
</tr>
<tr>
<td>References</td>
<td>23</td>
</tr>
</tbody>
</table>
Abstract

**Introduction:** Adhesive systems have revolutionized the practice of restorative dentistry due to their bonding to enamel and dentin. Recently, a new type of one-step self-etch adhesive has been introduced. This type of self-etch adhesive is classified as “universal” or “multi-mode” as they can be applied either with the etch-and-rinse or the self-etch technique.

**Purpose:** The goal of this study was to compare the microtensile dentin bond strength of a universal adhesive (Scotchbond™ Universal Adhesive) with or without an additional hydrophobic resin coating.

**Materials and methods:** Flat dentin surfaces were prepared in twelve non-carious human molars. Exposed dentin surfaces were wet-grounded with 200-, 400- and 600-grit silicon-carbide sandpaper to create a standardized smear layer. The teeth were equally and randomly divided in two groups. In group I dentin adhesive procedures were performed with a multimode adhesive system according to a self-etch approach (Scotchbond™ Universal Adhesive, SBU, 3M ESPE, St. Paul, MN, USA). In group II bonding procedures started with the same way but were followed by placing an extra hydrophobic resin layer (Adper™ Scotchbond™ Multi-Purpose, 3-Adhesive, 3M ESPE, St. Paul, MN, USA). After adhesive procedures, a 5-mm thick composite “restoration” was built over the bonded surface. Following storage in distilled water at 37 °C, the samples were cross-sectioned and sticks with 1,00mm² area were obtained and tested in tension in a universal testing machine at 0,5 mm/min. Data was analysed by the IBM SPSS software. The mode of failure was evaluated with optical microscopy.

**Results:** The following microtensile bond strengths were registered (mean in MPa ± Standard Deviation): Group I - 52,49 ± 13,56; Group II - 59,25 ± 12,39. The group II, coated with an extra hydrophobic resin layer had higher values in microtensile bond strength test than the group I, with statistically significant differences between them.

**Conclusion:** The use of an additional hydrophobic resin coating may increase the microtensile dentin bond strength of the Scotchbond™ Universal Adhesive.

**Keywords:** Microtensile; dentin bond strength; universal adhesives; hydrophobic resin coat; self-etch.
Influence of a hydrophobic resin coating on the microtensile dentin bond strength of a universal adhesive: in vitro study

Introduction

Adhesive systems have revolutionized the practice of restorative dentistry due to their bonding to enamel and dentin.\(^1\) The trend in recent developments resulted in simplified materials that are faster and easy to use but not necessarily better concerning effectiveness.\(^1\)

The main and global challenge for an adhesive system is the ability to bond effectively to two substrates of a different nature.\(^1\), \(^2\) Bonding to enamel is reliable and durable. In contrast, bonding to dentin becomes more difficult due to its variable nature and heterogeneous structure.\(^2\)

Contemporary dental adhesives systems can be classified according to the application techniques as etch-and-rinse and self-etching adhesive systems, including versions with three (only for the etch-and-rinse systems), two or one application step.\(^3\), \(^4\)

When using the etch-and-rinse strategy, the first step involves the application of a phosphoric acid gel to both dental substrates, which allows removal of the smear layer, tissue demineralization, exposure of the collagen fibrils in dentin, and increase in surface area and surface energy in the enamel substrate. The primer is then applied separately (second step) followed by the bonding resin (third step - three-step systems), or both products can be applied in a single solution and step (two-step systems).\(^4\) Irrespective of the number of steps, one disadvantage of the etch-and-rinse systems, mainly two-step versions, is that there is risk of collagen fiber collapse during the process of demineralized dentin drying, which leads to a decrease in bond strength.\(^4\) The collagen collapse can be prevented by keeping demineralized dentin sufficiently, but not excessively, moist, which is a difficult task to perform clinically.\(^5\) In fact, concerning dentin substrate, the over-wet and over-dry should be avoided because their negative effects in the adhesive interface quality.\(^5\) Adequate moisture depends on both the solvent used in the material and on the clinician’s interpretation of the manufacturer’s directions.\(^4\) Other disadvantage of these systems is the difference between the depth of dentin demineralization promoted by the phosphoric acid and the depth of dentin impregnation (hybridization) achieved by the resin monomers of the adhesive.\(^5\)

The incomplete impregnation of collagen fibers and the need to protect them against the degradation mechanisms present in the oral cavity environment, led to the development of the second category, the self-etch adhesive strategy.\(^4\) In this approach, there is no need to apply preliminary phosphoric acid gel as dentin demineralization and priming occurs simultaneously.\(^4\), \(^6\) Because no rinse procedure is applied, the dissolved hydroxyapatite crystals and residual smear layer are incorporated in the hybridized complex.\(^4\), \(^6\), \(^7\) At the same time they etch the dentin, they also, and simultaneously, infiltrate the exposed collagen
with hydrophilic monomers, and then copolymerize with the placed adhesive resin, always at the same dentin depth. The result is the formation of a hybridized complex, a hybridized smear layer and hybrid layer whose thickness is related to the characteristics of the self-etching agent. The whole extension of the demineralized dentin depth is impregnated by resin monomers, which may be the reason why self-etch systems are not related with the technique sensitivity characteristic of bonding to moist etched dentin. This advantage makes self-etch materials suitable for areas where adequate control of moisture is rather difficult, such as in posterior restorations. A clear disadvantage of the self-etch protocol is the reduction in enamel bonding effectiveness. This drawback is overcome using these adhesives with a selective enamel etching technique with phosphoric acid.

Therefore, it is clear that the adhesive technology tends to simplify bonding procedures by reducing application steps, shortening clinical application time and decreasing technique sensitivity, thus improving its standardization.

Aiming to provide a single product for all situations, “universal adhesives” or “multi-mode adhesives” have been more recently introduced and can be applied either with the etch-and-rinse or the self-etch mode. This multi-approach capability enables the clinician to apply the adhesive with the so-called selective enamel etching technique that combines the advantages of the etch-and-rinse technique on enamel, with the simplified self-etch approach on dentine, with additional chemical bonding on remnant carbonated apatite crystallites in those bonding substrates.

At present, there is only sparse literature reporting on the efficacy of universal adhesives. Similar adhesive performance has been observed for these adhesives regardless of their application mode, although bond strength degradation has been observed after ageing for the etched-and-rinse samples.

The increased amount of solvents and hydrophilic monomers in the adhesive formulations, namely in universal adhesives, lead to greater amount of residual solvents entrapped in the adhesive layer, which may reduce resin–dentin bond strengths, and increase the permeability of the adhesive layer after polymerization. One method suggested to surpass these problems includes the application of an additional layer of a hydrophobic resin coating over the polymerized simplified adhesive. This extra resin coat aims increasing the thickness and uniformity of the adhesive layer, as well as to reduce the fluid flow across the adhesive interface.

The development of a large number of this new type of adhesive systems available on the market imposes evaluation of their effectiveness. Thus, the aim of this study was to
compare the microtensile dentin bond strength of a universal adhesive employed in the self-etch approach (Scotchbond™ Universal Adhesive) with or without an additional hydrophobic resin coating.

The null hypothesis was that the application of an additional hydrophobic resin coat over this universal adhesive has no influence in the microtensile dentin bond strength.
Influence of a hydrophobic resin coating on the microtensile dentin bond strength of a universal adhesive: *in vitro* study

Materials and methods

Specimen preparation

Twelve non-carious human molars were collected and stored in distilled water for a period not exceeding six months after extraction. The teeth were cleaned from debris and partially included in a self-curing acrylic resin block (Vertex, Vertex-Dental, Zeist, Netherlands). The occlusal surfaces were cut perpendicularly to the long axis of the tooth (Accutom 5, Struers, Ballerup, Denmark), under water-cooling, thereby exposing a flat dentin surface without residual enamel. All occlusal surfaces were wet-ground with a sequence of 200-, 400- and 600-grit silicon-carbide sandpaper in circular motion for 60 seconds each one to obtain a uniform smear layer. The prepared occlusal surfaces were carefully observed to confirm the absence of residual enamel or other defects in dentin surfaces using a stereomicroscope (Nikon® SMZ 1500, Tokyo, Japan) at 20x.

Bonding and restorative procedures

The teeth were equally and randomly divided in two groups. In group I dentin adhesive procedures were performed with a multi-mode adhesive system in the self-etch mode (Scotchbond™ Universal Adhesive, SBU, 3M ESPE, St. Paul, MN, USA). In group II bonding procedures started with the same adhesive and were followed by placing an extra hydrophobic resin layer (Adper™ Scotchbond™ Multi-Purpose, 3-Adhesive, 3M ESPE, St. Paul, MN, USA), (Table I).

<table>
<thead>
<tr>
<th>Material</th>
<th>Manufacturer</th>
<th>Chemical Composition</th>
<th>Batch no.</th>
<th>Expiration date</th>
</tr>
</thead>
</table>
| Scotchbond™ Universal | 3M ESPE St Paul, MN, USA | - MDP phosphate monomer  
- Dimethacrylate resins  
- HEMA  
- Methacrylatemodified polyalkenoic acid copolymer  
- Filler  
- Ethanol  
- Water  
- Initiators  
- Silane | 551411 | 04/2016 |

Table I: Adhesive systems used in the study, manufacturers, chemical composition, batch numbers and expiration dates.
Influence of a hydrophobic resin coating on the microtensile dentin bond strength of a universal adhesive: in vitro study

Bis-GMA: Bisphenol A diglyciyl methacrylated; HEMA: 2-hydroxyethyl methacrylate; MDP: methacryloyloxydecyl.

The materials were applied as recommended by manufacturer (Table II). Light curing procedures were carried out using a LED light-curing unit (SPEC 3, Coltène, Altstätten, Switzerland).

**Table II: Adhesive application procedures in both groups.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Application Procedure</th>
</tr>
</thead>
</table>
| Group I (Without hydrophobic resin Coating) | 1. Apply the adhesive (Scotchbond™ Universal) to the entire preparation with a microbrush 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.                  |
| Group II (With hydrophobic resin Coating) | 1. Apply the adhesive (Scotchbond™ Universal) to the entire preparation with a microbrush 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.  
4. Apply a very thin layer of Adper™ Scotchbond™ Multi-Purpose, Adhesive, with a microbrush on the dental surface;  
5. Air blow to achieve an optimally thin layer;  
6. Light-cure for 10 s.                  |

Following bonding procedures, a light-cured nanofilled composite resin Filtek™ Z500 A3 (3M ESPE, St. Paul, MN, USA) was used to create resin-composite build-ups were in two 2.5 mm incremental layers (Table III). Each layer was initially light-cured for 10 seconds with a LED light-curing unit (SPEC 3, Coltène, Altstätten, Switzerland), followed by a final extra
polymerization time of 60 seconds (20 seconds on the occlusal surface and 10 seconds on the other surfaces). The specimens were then stored at 100% humidity at 37ºC for seven days (Heraeus BK 6160, Kelvitrón® Kp, Wehrheim, Germany).

Table III: Composite resin; manufacturer, composition, filler, batch number and expiration date.

<table>
<thead>
<tr>
<th>Composite</th>
<th>Manufacturer</th>
<th>Composition</th>
<th>Filler</th>
<th>Batch no.</th>
<th>Expiration date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtek™ Z500 A3</td>
<td>3M ESPE</td>
<td>Bis-GMA adduct</td>
<td>Zirconia/Silica cluster</td>
<td>475792</td>
<td>11/2015</td>
</tr>
<tr>
<td>Nanofilled</td>
<td>St Paul, MN, EUA</td>
<td>Bis-EMA adduct</td>
<td>Nanofiller silica (78,5wt %; 59,5vol %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>UDMA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TEGDMA</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bis-GMA: Bisphenol A dimethacrylate; Bis-EMA: Bisphenol A polyethylene glycol diether dimethacrylate; TEGDMA: Triethyleneglycol dimethacrylate; UDMA: Urethane dimethacrylate.

Cutting method

The specimens were cross-sectioned perpendicularly to the adhesive-tooth interface with a low-speed cutting saw (Accutom 5, Struers, Ballerup, Denmark), under water cooling at 300 rpm, according to the technique described by Sano et al.\textsuperscript{20}, to produce dentin-composite resin sticks with a sectional square area of 1,00 mm\textsuperscript{2}. After the first cut in x-axis direction, the free residual space between the slices was filled with light-bodied silicone Aquasil Ultra XLV (Dentsply, DeTrey, Konstanz, Germany). Then a second set of cuts were made in y-axis. Finally, the roots were cut from the crown approximately 3 mm below the cementoenamel junction releasing the dentin/composite sticks, which were then checked under an optical microscope (M300, Leica, Switzerland) at 40-fold magnification in order to exclude samples with enamel and defects. The number of stick specimens obtained per each group was: Group I (Without hydrophobic resin coating) n=42; Group II (With hydrophobic resin coating) n=45.
Microtensile bond strength testing

Each stick was bonded to a microtensile sampler holder with cyanoacrylate adhesive (Loctite Power Flex Gel, Henkel Ibérica S.A, Barcelona) and then fixed on the microtensile device (Od04-Plus; Odeme Dental Research, Luzerna, Brasil). Specimens were fractured in tensile mode in a universal testing machine (Model AG-1, Shimadzu Corporation, Kyoto, Japan) at a 0.5 mm/min speed and the maximum load (in MPa) at failure was recorded.

Figure 1: Schematic diagram from study.
After the microtensile testing, the fractured sticks were examined with an optical microscope (M300, Leica, Switzerland) at a 40-fold magnification and the mode of failure was recorded.

Failure modes were classified as: adhesive, if total failure occurred within the adhesive interface; cohesive, if complete failure occurred in the composite resin or in the dentin; and, finally, mixed, when simultaneously the adhesive and cohesive failure occurred.

**Statistical Analysis**

Statistical analysis was performed with IBM SPSS Statistics 20.0 (SPSS; Chicago, IL, USA). Student's t-Test for independent samples was used to compare means of microtensile bond strength data between the groups. The chi-square test was used to compare failure modes between the groups. Pearson Correlation test was used to compare the microtensile bond strength values with the failure modes in both groups. For all analysis the significance level was set at $\alpha = 0.05$. 
Results

Figure 2 and table IV shows the results of the microtensile bond strength of the two groups.

The Group II, coated with an extra hydrophobic resin layer, had a better performance in microtensile bond strength test than the Group I (Group I: 52.49 ± 13.56; Group II: 59.25 ± 12.39).

Table IV: Descriptive statistics for microtensile bond strength values of the two groups. Mean, standard deviation (SD), minimum and maximum values in MPa and lower/upper bound for mean at a 95% Confidence Interval.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean ± SD</th>
<th>Min</th>
<th>Max</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>42</td>
<td>52.49 ± 13.56</td>
<td>22.17</td>
<td>89.78</td>
<td>[48.26 ; 56.71]</td>
</tr>
<tr>
<td>II</td>
<td>45</td>
<td>59.25 ± 12.39</td>
<td>32.37</td>
<td>80.59</td>
<td>[55.52 ; 62.97]</td>
</tr>
</tbody>
</table>
Mean bond strength data for the two groups was compared using Student's t-Test for independent samples with a 95% confidence interval. The data was normally distributed for both groups (p>0.05), as assessed by Kolmogorov-Smirnov test. There was homogeneity of variances, as assessed by Levene’s Test of Homogeneity and Variance (p>0.05). Microtensile bond strength values were statistically significantly different between the groups. Therefore, as there was a statistically significant difference between means (p<0.05), thus we can reject the null hypothesis (Table V).

Table V: Comparison of mean bond strength data of the two groups (Student's t-Test for independent samples) with a 95% confidence interval. Mean difference of microtensile bond strength values between groups in MPa.

<table>
<thead>
<tr>
<th>p</th>
<th>Mean Difference</th>
<th>Std. Error Difference</th>
<th>95% Confidence Interval of the Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.017</td>
<td>-6.76</td>
<td>2.78</td>
<td>-12.29 to -1.23</td>
</tr>
</tbody>
</table>

The Table VI shows the distribution of the failure patterns of the experimental groups. There are not statistically significant differences in the distribution of the failure mode among groups, as evidenced by the Chi-square test and the more common failure mode was the cohesive in both groups. Besides that, there are not an association between the failure mode and the bond strength values, as assessed by the Pearson Correlation (p=0.413).

Table VI: Distribution of the failure patterns of the experimental groups. Absolute number of specimens (percentage).
Discussion

The development of a large number of new adhesive systems available on the market shows that adhesive dentistry is constantly changing. Universal or multi-mode adhesives represent the last generation of adhesives and they are designed under the “all-in-one” concept of already existing one-step self-etch adhesives, but incorporating the versatility of adapting them to specific clinical situations, by application under different etching modes. Considering all this progress, it is necessary to evaluate their effectiveness.

Bond strength is the force per unit required to break a bonded assembly with failure occurring in or near the adhesive interface. The bond strength is related to the size of the bonding area, therefore it is important to control specimens’ dimensions. By definition, the ideal bond strength test should be easy to perform, have low technique-sensitivity, be relatively fast, unsophisticated and inexpensive, and most important, capable to simulate biomechanics conditions of restored teeth.

Bond strength tests are capable of ranking dental adhesive systems according to their bond strength values. However, their results can be affected by several parameters, like, operator, adhesive system, substrate preparation, composite flexural modulus, testing conditions, among others. On the other hand, several factors can influence bond strength running in clinical situations, including masticatory stresses, pH and temperature cycling, as well as the wet environment, which might lead to fast degradation of the adhesive interface.

In the present study, it was used the microtensile bond strength test, which allowed measuring the tensile bond strength on very small surfaces, within an area around 1 mm². This method enables multiple specimens to be prepared from each tooth, measuring bond strength at critical areas, with a more uniform stress distribution in the reaction zone and a higher reliable correlation with clinical retention loss. In this test, the loading force passes through the tooth substrate and composite resin before the adhesive interface. Thus, the subsequent stress concentration could explain the frequent cohesive failure in the tooth substrate or material, which may underestimate the true bond strength.

Microtensile bond strength test have a number of advantages in relation to macro tests, such as: more adhesive failures and fewer cohesive failures; measurement of higher interfacial bond strengths; allows testing on very small surfaces or in irregular surfaces; means and variances can be calculated for single teeth; and facilitate examination of failed bonds by scanning electron microscopy. Still, some disadvantages were also described, as the labor intensity, technical demand and dehydration potential of these smaller samples. In addition, fixation of the specimen in the testing machine requires extreme careful manipulation as great loss of specimen can occur.
Scotchbond™ Universal is a one-step multi-mode adhesive system and in the present study had an excellent performance, achieving high microtensile dentin bond strength values for both groups tested. This adhesive system contains 10-methacryloyloxydecyl dihydrogen phosphate monomer (MDP) capable to chemically bond to calcium of hydroxyapatite, according to the adhesion decalcification concept, forming a stable calcium-phosphate at the adhesive interface. Among the currently used functional monomers, 10-methacryloyloxydecyl dihydrogen phosphate (MDP) has showed a very effective and durable bond to dentin, due to the low solubility of the calcium salt that forms on the hydroxyapatite surface, contributing for the success reported in the literature. Yoshida et al. (2012) showed that an effective chemical interaction occurs between MDP and hydroxyapatite forming a stable nano-layer that could form a stronger phase at the adhesive interface, which increases the mechanical strength of the adhesive interface. In addition, stable MDP-Ca salt deposition along with nano-layering may explain the high bond stability, which has previously been proven both in laboratory and clinical research.

Scotchbond™ Universal also contains the polyalkenoic acid copolymer, which in combination with MDP has shown contradictory results in the literature. Perdigão et al. (2012) reported higher microtensile dentin bond strength of Scotchbond™ Universal when compared to Clearfil™ SE Bond, which has only the MDP monomer, while Muñoz et al. (2013) observed a lower microtensile dentin bond strength of Scotchbond™ Universal when compared to the same other adhesive.

The polyalkenoic acid copolymer was firstly used in a commercially resin-modified glass-ionomer cement, Vitrebond™ and a chemical bond to the calcium in hydroxyapatite was also found for this compound. Clinical studies have shown a good performance of copolymer-containing etch-and-rinse adhesives, which may be attributed, at least partially, to chemical bonding. For self-etch adhesives, on the other hand, chemical bonding between polycarboxylic groups and hydroxyapatite plays a crucial role in the bonding mechanism. At least 2/3 of the carboxyl groups in the polyalkenoic acid copolymer are capable of bonding to hydroxyapatite. Carboxylic groups replace phosphate ions on the substrate and make ionic bonds with calcium. Adhesives that contain the polyalkenoic acid copolymer may even increase resistance to mechanical fatigue.

Although both molecules (MDP and polyalkenoic acid copolymer) may compete by binding to the calcium in hydroxyapatite, they are usually associated with improved adhesive performance, which may explain the good performance of Scotchbond™ Universal Adhesive in both bonding strategies (self-etch and etch-and-rinse).

Perdigão et al. (2012) evaluated the laboratory microtensile dentin bond strength of the Scotchbond™ Universal Adhesive applied under different bonding strategies and
generated a mean value of 54.4 ± 18.5 MPa when it was applied as a one-step self-etch adhesive. In addition, there were no statistical differences in microtensile dentin bond strength among the different adhesion strategies for Scotchbond™ Universal (self-etch or etch-and-rinse). Marchesi et al.12 (2014) investigated the adhesive stability over time of Scotchbond™ Universal Adhesive applied under different bonding techniques on human coronal dentin. The mean value obtained for immediate microtensile dentin bond strength with the self-etch strategy was 35.5 ± 9.7 MPa. For the etch-and-rinse technique the immediate bond strengths were 34.8 ± 9.4 MPa on wet dentin and 41.6 ± 10.3 MPa on dry dentin. After one year storage in artificial saliva at 37 °C the dentin bond strengths decreased to 26.8 ± 9.5 MPa in the self-etch mode application. This one-year value was higher than the one-year values obtained for Scotchbond™ Universal when the adhesive was applied using the etch-and-rinse technique either on wet (21.9 ± 9.5 MPa) or dry dentin (21.8 ± 9.4 MPa). Besides that, after one-year storage, the nanoleakage expression was lower when Scotchbond Universal™ was applied in the self-etch mode compared to the etch-and-rinse applications. Therefore, the results of this study support the use of the self-etch approach for bonding this adhesive to dentin due to improved stability over time. Wagner et al.8 (2014) compared the microtensile dentin bond strength of three universal adhesives (All-Bond Universal®, Futurabond® Universal and Scotchbond™ Universal) applied in two different etching modes (self-etch and etch-and-rinse) and obtained a mean value of 44.0 ± 21.9 MPa for the Scotchbond™ Universal when it was applied as a one-step self-etch adhesive. A conclusion of the study was that the application of an etching step prior to the bonding procedure did not affect the bond strength to dentin of the universal adhesives. Chen et al.36 (2015) examined the short-term in vitro performance of five universal adhesives (Prime&Bond Elect®, Scotchbond™ Universal, All-Bond® Universal, Clearfil™ Universal Bond and Futurabond® Universal) bonded to human coronal dentine. The use of each adhesive in either the etch-and-rinse or self-etch application mode did not result in significantly different microtensile dentin bond strength and the mean value obtained for Scotchbond™ Universal was 59.9 ± 11.8 MPa with the self-etch strategy and 55.7 ± 10.7 MPa with the etch-and-rinse strategy.

A systematic review and meta-analysis (2015), which included a total of ten manuscripts dated from 2012 to 2014, evaluated six different types of universal adhesives. Seven of the studies included evaluated the multi-mode adhesive Scotchbond™ Universal. This review demonstrated that prior acid etching did not influence the dentin bond strength for universal adhesives like Scotchbond™ Universal. However, in vitro literature highlights that enamel bond strength of universal adhesives is improved with prior phosphoric acid etching.23
In accordance with previously published literature, Scotchbond™ Universal shows good and consistent bond strength values to dentin, despite the differences found between the different studies. In fact, as above referred, variables such as specimen shape (stick, dumbbell, hourglass), flaws (e.g. air bubbles) in the adhesive or grinding flaws during specimen preparation, thickness of the adhesive, composite resins and substrate variability, operator, jigs and angle of load application will influence to various degrees the stress distribution in each specimen design and thus contribute to those variations in the bond strength results.37, 38

One-step self-etch adhesives are produced from a complex blend of hydrophilic and hydrophobic monomers, organic solvents and water in order to combine etching, priming, and bonding application steps into a one-bottle solution.39 One-step self-etch adhesives are hydrophilic in nature, allowing water to flow from dentin into the adhesive up to the adhesive/composite interface even after polymerization.6, 40, 41 The water affinity of this category of dentin adhesive can result in negative consequences to marginal sealing and bond strengths to dentin.40 Water tends to accumulate on the top surface of the hybrid layer, inhibiting copolymerization within the adhesive, modifying the polymers and accelerating degradation of the adhesive.42, 43 The adverse effect of one-step self-etch adhesive hydrophilicity on bonding to dentin has been reported.40, 44 In vitro dentin bond strength tests indicate that the lack of a hydrophobic bonding resin in one-step self-etch adhesive formulations, reduce bond stability over time, because the bonded interfaces behave as semi-permeable membranes allowing the movement of water across them and expediting hydrolytic degradation of one-step self-etch, contributing for reduced short-term and medium-term values.40 These assumptions can be confirmed by the conclusions of a review on the durability of adhesion to tooth structures.45

The application of an additional layer of hydrophobic resin over one-step self-etch adhesives has been suggested as an alternative procedure to surpass these drawbacks.40, 46 The beneficial effect of converting one-step self-etch adhesives into two-step self-etch adhesives, by applying an additional coat of a hydrophobic resin has been proven under laboratory conditions.18, 40, 44

Like any other one-step self-etch adhesive, Scotchbond™ Universal has a basic chemical composition that gives it a high hydrophilicity, potentially contributing to present the adverse effects and problems above reported.17

In the present study, it was found a significant improvement of immediate bond strengths to dentin when an extra hydrophobic resin layer was applied over the self-etch mode of the Scotchbond™ Universal Adhesive.
Muñoz et al.\textsuperscript{17} (2014) obtained a similar result once the use of an additional hydrophobic resin coating improved the adhesive performance in terms of resin-dentin bond strengths of three universal adhesives (including Scotchbond\textsuperscript{TM} Universal) when used with the self-etch strategy.\textsuperscript{17} For this adhesive system, the values for resin-dentin microtensile bond strength were 34.7 ± 5.8 MPa (without the coating) and 42.7 ± 6.1 MPa (with coating). Perdigão et al.\textsuperscript{47} (2014) also obtained a better performance in microtensile bond strength for another universal adhesive system (G-Bond\textsuperscript{TM} Plus), with the use of a hydrophobic resin coat.

The extra layer of hydrophobic resin adds unsolvated hydrophobic monomers to the adhesive interface, which decreases the relative concentration of retained solvents and unreacted monomers in the adhesive layer. This, in turn, increases the ultimate tensile strength of the adhesive interface, due to the formation of a more densely packed hybrid layers, making it more resistant to the tensile forces during microtensile bond strength testing and less prone to degradation effects over time.\textsuperscript{17, 48}

In fact, the increased immediate bond strengths in the present study, might be due to the increase in the adhesive thickness of the adhesive layer, which is known to reduce the detrimental effects of polymerization shrinkage of composites\textsuperscript{49} and improve stress distribution during testing. Besides that, the hydrophobic resin coating increases the hydrophobicity of the adhesive layer, rendering them less permeable to water movement, more compatible with the light-cured composite resin, and less susceptible to water degradation.\textsuperscript{44}

In the present study, there were not statistically significant differences in the distribution of the failure mode between the groups and also there was not a statistically significant association between the failure mode and the bond strength values. Different results were reported in other study. Ceballos et al.\textsuperscript{50} (2003) correlated the bond strength values with mode of failure where low bond strengths were associated with adhesive failures, while cohesive fractures were seen at higher bond strengths.

Another study accomplished by Scherrer et al.\textsuperscript{38} (2010) stated that the common cohesive failures that occurred in microtensile bond strength tests may be due to the errors in the alignment of the specimen along the long axis of the testing device or to the introduction of microcracks during cutting. In the present study cohesive failures occurred frequently. Concerning this point, it can be understood that, at least, the adhesive bonding to dentin was stronger than the registered value and therefore of the cohesive strength of composite or dentin.
Concerning future research perspectives, it will be valuable improving the standardization of test conditions, studying the same adhesives with different methodologies and even with different operators, as well as conducting in vitro tests after aging, complemented with clinical trials. Furthermore, researches evaluating other bonding properties and different adhesive bonding approaches should be targeted.
Conclusion

Within the limitations of this in vitro study, it can be concluded that the use of an additional hydrophobic resin coating improved the adhesive performance, in terms of resin-dentin bond strength, of Scotchbond™ Universal Adhesive.
Acknowledgements

Ao meu orientador, Prof. Doutor João Carlos Ramos. Os seus amplos conhecimentos nesta área orientaram-me neste trabalho e sem ele o mesmo não teria sido possível. A dedicação e empenho que demonstrou durante todo este ano, constituem um exemplo que procuro seguir durante a minha vida profissional. Um Bem-haja!

À Doutora Alexandra Vinagre, o meu sincero agradecimento pela coorientação deste projeto. Muito obrigado pela sua total disponibilidade, pela constante simpatia, pela confiança depositada em mim e por todos os conhecimentos que me transmitiu durante a realização deste trabalho.

À Drª. Ana Messias pela ajuda na análise estatística dos resultados e à D. Cláudia Brites por toda a ajuda prestada e pela paciência inesgotável.

Ao Prof. Doutor José Maria Ferreira e ao Eng. Bruno Almeida do departamento de Engenharia de Materiais e Cerâmica da Universidade de Aveiro, pela ajuda concedida na realização deste trabalho, nomeadamente durante os procedimentos experimentais.

A todos os Professores do Mestrado Integrado em Medicina Dentária pelos conhecimentos transmitidos e por todo o apoio demonstrado durante este trajeto. Foi um privilégio frequentar este curso, nesta Faculdade, que muito contribuiu para o enriquecimento da minha formação académica e cívica.

A todos os meus amigos que me acompanharam durante este percurso, um grande obrigado por toda a sinceridade, companheirismo e acima de tudo amizade que demonstraram. Não posso deixar de agradecer especialmente à Bárbara por todos os momentos fantásticos que partilhou comigo durante estes anos. Um enorme obrigado por todo o carinho, pelo constante apoio e ajuda (também na realização deste trabalho), pela paciência e pela compreensão que sempre demonstrou e acima de tudo por ser a pessoa maravilhosa que é. Ao Renato e ao Ricardo, por me terem conseguido aturar durante estes 5 anos. Imagino que nem sempre tenha sido fácil por isso obrigado pela amizade e por toda a ajuda que me deram. Aos meus colegas de casa, Diogo, Eduardo e Rosa pelas aventuras vividas e pelos bons momentos que com eles partilhei. Obrigado pela amizade e companhia.

Ao meu cunhado, um grande obrigado pela amizade e disponibilidade que sempre demonstrou para comigo.

E por último, agradeço à minha família, nomeadamente aos meus Pais e Irmãos por todos os ensinamentos e valores que me inculiram durante a minha vida. Um enorme obrigado por acreditarem sempre em mim e naquilo que faço. Espero sinceramente que após terminar esta etapa da minha vida, possa de alguma forma retribuir e compensar todo o carinho, apoio e dedicação que, diariamente, me oferecem.
References


