

Universidade de Lisboa
Faculdade de Medicina Dentária



***In vitro* microtensile bond strength of a
Universal Adhesive System to dentin using
Etch-and-Rinse and Etch-and-Dry technique**

Sara Palmares Fernandes

Dissertação
Mestrado Integrado em Medicina Dentária

2014

Universidade de Lisboa
Faculdade de Medicina Dentária



***In vitro* microtensile bond strength of a
Universal Adhesive System to dentin using
Etch-and-Rinse and Etch-and-Dry technique**

Sara Palmares Fernandes

Dissertação orientada pela Dra. Ana Pequeno
e co-orientada pela Dra. Ana Catarina Coito

Mestrado Integrado em Medicina Dentária

2014

*“Para ser grande sê inteiro: nada
Teu exagera ou exclui.
Sê todo em cada coisa; Põe quanto és
No mínimo que fazes.
Assim em cada lago, a lua toda
Brilha, porque alta vive.”*

Fernando Pessoa

Agradecimentos

À minha orientadora, Dra. Ana Pequeno, pelo constante apoio, paciência e disponibilidade que sempre demonstrou perante as minhas dúvidas e nas inúmeras vezes que reviu a minha tese. Por me ter introduzido no mundo da Adesão Dentária e da Microtração e partilhado comigo todo o seu conhecimento.

À minha co-orientadora, Dra. Catarina Coito, por me ter transmitido o gosto pela investigação, pela constante disponibilidade e entusiasmo contagiante nas horas laboratoriais.

Ao Departamento de Dentisteria Operatória por toda a ajuda prestada e pela forma alegre com que brindaram todas as horas laboratoriais. Ao Prof. Dr. Alexandre Cavalheiro, mentor deste projeto.

Ao Prof. Dr. Luís Pires Lopes pela disponibilização das instalações, materiais e equipamentos do Laboratório de Biomateriais da Faculdade de Medicina Dentária da Universidade de Lisboa.

Aos meus queridos amigos, Joana Costa, Inês Marcos, Margarida Simões, Daniela Rocha, Ruben Pereira e David Braz, pela sua amizade durante estes cinco anos e por terem partilhado comigo os bons e os maus momentos.

Aos meus amigos de infância, Ricardo Santos, Pedro Ribeiro, Marta Didier e Nadja Leite, por estarem presentes em todos os momentos da minha vida.

À minha família por tudo, e em especial aos meus Pais e Irmã, pelo amor incondicional, motivação e apoio em todas as etapas da minha vida.

A todos, o meu sincero obrigada

Table of Contents

Abstract	xi
Resumo	xiii
I. Introduction	1
1. Classification of contemporary adhesives	2
1.1 Total-etch or Etch-and-rinse adhesives.....	2
1.2 Self-etch or Etch-and-dry adhesives	3
2. Microtensile bond strength test.....	5
II. Materials and Methods	7
1. Type of study	8
2. Design of the study	8
3. Teeth preparation	8
4. Distribution and treatment of the crown segments	11
5. Specimens preparation for the microtensile tests.....	13
6. Microtensile tests	14
7. Statistical Analysis.....	15
III. Results	17
IV. Discussion	21
V. Conclusions	29
VI. Appendices	I
APPENDIX 1	II
APPENDIX 2	III
APPENDIX 3	IV
APPENDIX 4	V
APPENDIX 5	VI
VII. References	VII

List of tables and figures

Table 1 - Tests of Normality of the μ TBS in MPa for the two different experimental groups tested.	15
Table 2 - Test for Equality of Variances of the μ TBS in MPa.	15
Table 3 - Descriptive statistics of the μ TBS in MPa for the two experimental groups tested.	18
Table 4 - t-test for Equality of Means ($p \leq 0,05$).	18
Table 5 - Number of specimens according to the failure mode and premature failures of the two different experimental groups tested.	19
Table 6 - Number of specimens according to the failure mode and premature failures of all experimental groups tested.	20
Table 7 - Materials, Manufacturer, Composition and Batch Numbers.	II
Table 8 - Application mode of Scotchbond Universal Adhesive according to the manufacturer's instructions.	III
Table 9 - Application mode of Scotchbond Universal Adhesive according to the detailed manufacturer's instructions.	IV
Table 10 - Number of sticks obtained in each tooth.	VI
Figure 1 - Diamond wafering blade.	8
Figure 2 - Isomet 1000 Precision Saw, hard tissue microtome.	8
Figure 3 - Tooth attached to an acrylic holder with sticky wax.	9
Figure 4 - Roots cutting 1-2 mm below CEJ.	9
Figure 5 - Exposure of the pulp chamber after roots cut.	9
Figure 6 - Removal of the pulp tissues.	9
Figure 7 - Filling of the pulp chamber with cyanoacrylate glue.	9
Figure 8 - The crown fixed to the acrylic holder.	10
Figure 9 - Removal of the occlusal enamel and superficial dentin.	10
Figure 10 - Mid coronal dentin surface.	10
Figure 11 - Lunn Major, mechanical grinder.	10
Figure 12 - Scotchbond Universal Adhesive and Scotchbond Universal Etchant.	12
.....	
Figure 13 - Resin composite ENAMEL Plus HRi.	12

Figure 14 - Resin composite build-up.	12
Figure 15 - The teeth after being sectioned in both “x” and “y” directions.	13
Figure 16 - The sticks obtained after the final cut.	13
Figure 17 - The specimen attached to a Geraldelli’s jig.	14
Figure 18 - Instron 4502, universal testing machine.	14
Figure 19 - Box-whisker plots of the μ TBS in MPa for the two different experimental groups tested.	18
Figure 20 - Distribution of the specimens according to the failure mode of the two different experimental groups tested.	19
Figure 21 - Distribution of the specimens according to the failure mode of all experimental groups tested.	20
Figure 22 - Cyanoacrylate glue.	V
Figure 23 - Halogen light-activation unit.	V
Figure 24 - Incubator.	V
Figure 25 - Radiometer.	V
Figure 26 - Digital caliper.	V
Figure 27 - Stereomicroscope.	V

Abbreviates

ED	Etch-and-dry
ER	Etch-and-rinse
HAp	Hydroxyapatite
10-MDP	10-methacryloyloxydecyl dihydrogen phosphate
4-MET	4-methacryloyloxyethyl trimellitic acid
MMP-2	Matrix metalloproteinase-2
MMP-8	Matrix metalloproteinase-8
MMP-9	Matrix metalloproteinase-9
PA	Polyalkenoic acid copolymer
Phenyl-P	2-(methacryloyloxyethyl)phenyl hydrogenphosphate
Ptf	Pre-testing failures
SBU	Scotchbond Universal
SE	Self-etch
TE	Total-etch
UA	Universal adhesives
μ TBS	Microtensile bond strength

Abstract

Objectives: The purpose of the present study is to compare the immediate microtensile bond strengths (μ TBS) of one universal adhesive applied to dentine according to the etch-and-rinse and the self-etch technique. The null hypothesis tested was that the bonding effectiveness to dentin was not affected when the adhesive was applied either following an etch-and-rinse or etch-and-dry technique.

Methods: Six recently extracted human third molars, intact and without macroscopic evidence of caries or restorations, were assigned to two groups according to the etching strategy: Group SBU TE D – Scotchbond Universal Adhesive (3M ESPE) applied as a 2-step etch-and-rinse adhesive on moist dentin; Group SBU SE D - Scotchbond Universal Adhesive applied as a 1-step self-etch adhesive on dentin. After resin composite build-ups were performed, the teeth were stored in distilled water in an incubator for 24 hours at 37°C. Specimens were sectioned with a slow-speed diamond saw under water irrigation to obtain bonded sticks that were tested to failure in a universal testing machine at a crosshead speed of 1mm/minute. The statistical analysis of the results was performed with SPSS. A paired-sample t-test was performed when the assumption of normality was valid.

Results: The mean μ TBS to dentin of the Scotchbond Universal Adhesive applied following the etch-and-dry approach (SBU SE D) was statistically higher than that when the SBU was applied following the etch-and-rinse approach (SBU TE D) ($p = 0,008$).

Conclusions: Within the limitations of the present laboratory study, it may be concluded that improved bonding effectiveness of SBU on dentin is obtained when the adhesive is applied with the self-etch approach. The etch-and-rinse approach tested (wet dentin) resulted in immediate bond strength statistically lower than the self-etch group.

Keywords: Dental adhesives, universal adhesives, dentin bonding, microtensile bond strengths.

Resumo

Durante as últimas décadas, a adesão dentária tem adquirido uma importância cada vez maior. O principal desafio dos sistemas adesivos amelodentinários é providenciar uma adesão eficaz a tecidos duros de diferente natureza. Desde os trabalhos de Buonocore, a adesão ao esmalte permaneceu simples e consistente ao longo dos anos. Pelo contrário, a adesão à dentina revelou-se imprevisível devido às suas características morfológicas, levando à sucessiva modificação dos sistemas adesivos.

O mecanismo base da adesão, tanto ao esmalte, como à dentina, é essencialmente um processo que envolve a substituição da porção mineralizada destes tecidos pelos monómeros da resina adesiva, que se unem micromecanicamente às porosidades criadas pelo condicionamento ácido. As fragilidades inerentes a este mecanismo, em especial na dentina, devem-se à complexidade da técnica e à sua sensibilidade. Consequentemente, a exigência por sistemas adesivos mais simples, com um menor número de passos e uma técnica menos sensível, é cada vez maior, levando os fabricantes a desenvolverem novos adesivos num curto período de tempo.

Apesar dos sistemas adesivos mais simplificados apresentarem uma longevidade comprometida tanto *in vitro*, como *in vivo*, comparativamente com aqueles que se baseiam em diversos passos, alguns fabricantes introduziram recentemente uma classe de adesivos mais versátil, desenvolvida para simplificar e reduzir o tempo de aplicação clínico. Estes novos adesivos podem ser aplicados segundo a técnica *etch-and-rinse* (2 passos) ou *etch-and-dry* (1 passo). Para os adesivos *etch-and-rinse*, a força adesiva provém da formação da camada híbrida. Contrariamente, para os adesivos *etch-and-dry*, a adesão química tem mostrado ter um papel mais importante. Os adesivos *mild self-etch* têm como mecanismo de adesão uma combinação entre a adesão química à hidroxiapatite e a adesão micromecânica.

Objetivos: Um único sistema adesivo que possa ser aplicado de diferentes formas e que permita ao clínico escolher qual a estratégia adesiva mais adequada para a cavidade preparada, é desejável. Estes novos adesivos são chamados de Adesivos Universais, ou “*Multi-purpose*” ou “*Multi-mode*”. Devido ao fato destes adesivos serem novos no mercado e do respetivo conceito ser recente, a informação existente na literatura sobre o desempenho *in vitro* e *in vivo* desta nova categoria de adesivos é escassa. Por esta razão, o objetivo deste estudo laboratorial é comparar as forças de adesão à dentina de um

adesivo universal, segundo a estratégia *etch-and-rinse* e *etch-and-dry*, através de testes de microtração (μ TBS) (Scotchbond Universal Adhesive, 3M ESPE, Neuss, Germany). A hipótese nula deste estudo é: não há diferenças nas forças de adesão à microtração quando o adesivo é aplicado segundo a técnica *etch-and-rinse* ou *etch-and-dry*.

Materiais e métodos: Uma amostra conveniente composta por seis terceiros molares humanos recentemente extraídos, intactos e sem evidência macroscópica de cárie ou restaurações (n=6), foi distribuída aleatoriamente em dois grupos, segundo a estratégia de adesão: Grupo SBU TE D - Scotchbond Universal Adhesive aplicado como um adesivo *etch-and-rinse de 2 passos* (em dentina húmida); Grupo SBU SE D - Scotchbond Universal Adhesive aplicado como um adesivo *etch-and-dry* de 1 passo.

Após a preparação dos dentes e, com o objetivo de formar uma *smear layer* semelhante à que é obtida em situações clínicas, a superfície dos dentes foi polida com papel abrasivo de carbureto de silício (SiC) de grão 600, durante 60 segundos sob refrigeração com água, numa máquina polidora (Lunn Major, Struers, Denmark). Procedeu-se de seguida à aplicação do sistema adesivo de acordo com a distribuição nos respetivos grupos experimentais. Em todas as amostras o adesivo foi fotopolimerizado durante 10 segundos com o fotopolimerizador ELIPAR S10 (3M ESPE Seefeld, Germany) com intensidade de 600 mW/cm^2 , controlado periodicamente por um radiómetro (Curing Radiometre P/N 10503, USA). De seguida foi aplicada a resina composta ENAMEL Plus HRi (Micerium S.p.A. Avegno (GE) Italy) em camadas de aproximadamente 2 mm e fotopolimerizadas entre si durante 20 segundos, de acordo com as instruções do fabricante. As faces vestibular, lingual, mesial e distal foram polimerizadas adicionalmente por mais 10 segundos cada. A face exterior da resina composta de todos os espécimes foi pintada com tinta à prova de água, com o objetivo de excluir do estudo os palitos nos quais a adesão foi feita em esmalte. Os dentes foram armazenados em água destilada numa estufa de incubação durante 24h a 37°C e registado o dia e a hora da reconstrução.

Posteriormente, foram efetuados cortes no eixo do “x” e do “y” com um disco de diamante, a baixa rotação e sob refrigeração com água, num micrótomo de tecidos duros (Isomet 1000 Precision Saw, Buehler Ltd., Lake Buff, IL, USA), com o objetivo de obter palitos com uma área de, aproximadamente, $0,7 \text{ mm}^2$. Cada palito foi colado individualmente num suporte de Geraldelli’s, com cola de cianoacrilato, e testados um a um. Foram sujeitos a forças de tração numa máquina de Teste Universal, a uma velocidade de 1 mm/minuto até ocorrer fratura. Mediu-se a secção de cada espécimen

fraturado com uma craveira digital e determinou-se a área em milímetros quadrados (mm^2). A área de superfície de cada palito e a sua resistência à fratura, medida em KiloNewtons (KN), foram registradas e, a partir delas, calculadas as forças de adesão em MegaPascals (MPa). Cada fratura foi observada ao estereomicroscópio (Nikon, Japan) com uma ampliação de 10x, para se caracterizar o tipo de fratura ocorrida (coesiva, adesiva ou mista). Quando a fratura ocorreu exclusivamente na dentina foi denominada como coesiva de dentina (CD) e quando ocorreu exclusivamente na resina composta foi classificada como coesiva de compósito (CC). Quando a fratura ocorreu na interface dentina-adesivo, denominou-se de adesiva (A) e, quando atingiu tanto a dentina como a resina composta, foi denominada como mista (M).

A análise estatística dos resultados foi realizada através do teste paramétrico emparelhado *t-test*, após se ter verificado que a amostra seguia uma distribuição normal (os testes de Kolmogorov-Smirnov e Shapiro-Wilk foram usados para avaliar se os resultados seguiam uma distribuição normal; o teste de Levene foi usado para determinar a igualdade de variâncias). O intervalo de confiança definido foi de 95%. O número de palitos fraturados durante a sua preparação (palitos descolados) foi registrado, no entanto, esses valores não foram considerados para a análise estatística.

Resultados: Foram testados 140 palitos, 68 correspondentes ao grupo SBU TE D e 72 correspondentes ao grupo SBU SE D. As forças de adesão quando o SBU foi aplicado segundo a técnica *self-etch* (SBU SE D) ($56,9 \pm 2,5$ MPa) foram superiores às forças de adesão quando o SBU foi aplicado segundo a técnica *total-etch* (SBU TE D) ($48,0 \pm 2,1$ MPa). A análise estatística com o *t-test* determinou a existência de diferenças estatisticamente significativas entre estes grupos ($p=0,008$). Após a observação do tipo de fratura ocorrida, verificou-se que, no total dos dois grupos, 49,3% foram fraturas adesivas, 7,8% mistas, 17,9% coesivas de dentina e 25% coesivas de compósito.

Conclusões: Tendo em conta as limitações deste estudo laboratorial, pode-se concluir que o SBU apresenta uma melhor eficácia na dentina quando aplicado segundo a técnica *self-etch*. A técnica *total-etch* (em dentina húmida) resultou em forças adesivas imediatas estatisticamente inferiores ao grupo *self-etch*.

Estudos futuros poderão avaliar o efeito a longo prazo do armazenamento em água no desempenho deste adesivo. Além disso, estudos em diferentes substratos, como por exemplo, em dentina cariada, em associação com estudos *in vivo* são necessários para avaliar o desempenho clínico desta nova classe de adesivos, para que possam ser utilizados futuramente com maior conhecimento.

Palavras-chave: Adesivos dentários, adesivos universais, adesão à dentina, forças de adesão à microtração.

I. Introduction

During the last decades, restorative concepts have been continually changing, and adhesive technology has become steadily more important. Adhesion to enamel was followed by adhesion to dentin. Initially simply designed “bonding agents” gradually evolved to multi-step “adhesive systems” with rather complicated application procedures (Swift *et al.*, 1995; Van Meerbeek *et al.*, 1998). The main challenge for dental adhesives is to provide an equally effective bond to two hard dental tissues of different nature. Bonding to enamel has been proven to be durable. Bonding to dentin is far more intricate and can apparently only be achieved when more complicated and time-consuming application procedures are followed (Swift *et al.*, 1995; Perdigao *et al.*, 2000; Van Meerbeek *et al.*, 2011). Consequently, today’s adhesives are often regarded as technique-sensitive with the smallest error in the clinical application procedure being penalized either by rapid debonding or early marginal degradation (De Munck *et al.*, 2005a; Breschi *et al.*, 2008; Van Meerbeek *et al.*, 2011). As a consequence, the demand for simpler, more user-friendly and less technique-sensitive adhesives remains high, urging manufacturers into developing new adhesives at a rapid pace.

1. Classification of contemporary adhesives

The bonding mechanism of adhesive systems basically involves the replacement of minerals removed from the hard dental tissue by resin monomers, in such a way that a polymer becomes micro-mechanically interlocked to the dental substrate (Nakabayashi *et al.*, 1982). However, the adhesive systems available on the market can be classified into two categories, in function of how the adhesive interacts with the smear layer: etch-and-rinse (ER) or total-etch (TE) and etch-and-dry (ED) or self-etch (SE) adhesives (Van Meerbeek *et al.*, 1998; De Munck *et al.*, 2005a).

1.1 Total-etch or Etch-and-rinse adhesives

When using the ER strategy, the first step involves the application of phosphoric acid gel to both dental substrates, which allows removal of the smear layer, exposure of the collagen fibrils in dentin, and increase in surface area and surface energy in the enamel substrate (Nunes *et al.*, 2001; Perdigao & Lopes, 2001). This conditioning step is followed by a priming step and application of the adhesive resin, resulting in a three-step procedure. Simplified two-step ER adhesives combine the primer and adhesive resin into one

application (Van Meerbeek *et al.*, 1998; Van Meerbeek *et al.*, 2003; De Munck *et al.*, 2005a).

Irrespective of the number of steps, the main disadvantage of the ER system, mainly two-step version, is that there is risk of collagen fibre collapse during the process of demineralized dentin drying, which leads to a decrease in bond strength (Pashley *et al.*, 1992; Swift *et al.*, 1995; Hashimoto *et al.*, 2000; Perdigao *et al.*, 2002; De Munck *et al.*, 2009). The collagen collapse is prevented by keeping demineralized dentin moist, which is a difficult task to perform clinically. In fact, adequate moisture depends on both the solvent used in the material and on the clinician's interpretation of the manufacturer's directions (Pashley *et al.*, 2011; Munoz *et al.*, 2013).

The incomplete impregnation of collagen fibers by the adhesive monomers can lead to the degradation of the exposed collagen fibrils within the hybrid layers by endogenous proteases enzymes present in dentin (collagenase MMP-8 and gelatinases MMP-2 and -9) (De Munck *et al.*, 2005a; Pashley *et al.*, 2011; Mazzoni *et al.*, 2013). The need to protect them against the degrading mechanisms present in the oral cavity environment (Perdigao & Lopes, 2001; De Munck *et al.*, 2005a), led to the development of the second category, an adhesive using the self-etch strategy (Pashley *et al.*, 2011; Munoz *et al.*, 2013).

1.2 Self-etch or Etch-and-dry adhesives

In the ED strategy, there is no need to apply a preliminary phosphoric acid gel on dental substrates. Dentin demineralization and priming occur simultaneously, based on the use of non-rinse acidic monomers dissolved in an aqueous solution (Van Meerbeek *et al.*, 2003; De Munck *et al.*, 2005a). The goal is to incorporate the smear layer into the hybrid layer (Tay & Pashley, 2001). This approach has been claimed to be user-friendly (shorter application time, less steps) and less technique-sensitive (no wet-bonding, simple drying) (Pashley & Tay, 2001; Tay & Pashley, 2001; Van Meerbeek *et al.*, 2003; Yoshida *et al.*, 2004). Another important advantage of the self-etch is that the infiltration of resin occurs simultaneously with the self-etching process to the same depth, by which the risk of discrepancy between both processes is low or non-existent. Therefore, collapse of air-dried, demineralized collagen is prevented (Tay & Pashley, 2001; Van Meerbeek *et al.*, 2003). This should be attributed to their less aggressive and thus more superficial interaction with dentin (Van Meerbeek *et al.*, 2011).

Even if all ED adhesives systems rely on the same bonding mechanism, they differ from each other in many aspects, such as acidic resin monomer composition, water content and acidity. Thus, these adhesives may be further classified according to the pH of the adhesive solution as mild ($\text{pH} > 2$), intermediately strong ($1 < \text{pH} < 2$) or strong ($\text{pH} \leq 1$). Indeed the adhesive acidity influences the ability of the system to interact with the underlying enamel and dentin (Van Meerbeek *et al.*, 2003). The high acidity of strong self-etch adhesives results in rather deep demineralization effects. Consequently, the underlying bonding mechanism is primarily diffusion-based, similar to the etch-and-rinse approach (Van Meerbeek *et al.*, 2003; Yoshida *et al.*, 2004). Mild self-etch systems demineralize dentin only to a depth of 1 μm . This superficial demineralization occurs only partially, keeping residual hydroxyapatite still attached to exposed collagen. Specific carboxyl or phosphate groups of functional monomers (4-MET, phenyl-P and 10-MDP) can then chemically interact with this residual hydroxyapatite (Yoshida *et al.*, 2000; Van Meerbeek *et al.*, 2003; Yoshida *et al.*, 2004). This chemical bond seems to be important in stabilizing the adhesive interface on dentin over time (Van Meerbeek *et al.*, 2011). Nevertheless, sufficient surface-porosity is created to obtain micromechanical interlocking through hybridization. The thickness of the hybrid layer is, however, much smaller than that produced by the strong self-etch or etch-and-rinse approach (Van Meerbeek *et al.*, 2003).

Self-etch adhesives can come as “two-step” or “self-etching primers” and “one-step” adhesives, depending on whether a self-etching primer and adhesive resin are separately provided or combined into one single solution. One-step adhesives can be further divided into two-component one-step self-etch adhesive (by separating active ingredients), and single-component one-step self-etch adhesives, that are considered the only true “one-bottle” or “all-in-one” adhesives (Van Meerbeek *et al.*, 2003; Van Meerbeek *et al.*, 2011).

“All-in-one” must today be regarded as the most simplified adhesive protocol that can clinically be employed to bond composite to tooth enamel and dentin, combining all bonding steps into one single application step. Concerns remain regarding both their short- and long-term bonding effectiveness (Frankenberger *et al.*, 2001). In terms of immediate performance these simplified one-step adhesives showed promising results (De Munck *et al.*, 2003). However, the long-term performance is inferior in terms of bond durability, in particular when compared to the gold-standard three-step etch-and-rinse approach (Breschi *et al.*, 2008; De Munck *et al.*, 2012; Marchesi *et al.*, 2013).

2. Microtensile bond strength test

The popularity of laboratory studies in the field of adhesive dentistry may in part be ascribed to the rapid evolution of dental adhesive technology and the resultant high turnover of adhesive systems, which often tempts manufacturers to release a successor product on the market even before its precursor has been clinically evaluated, at least in the long term (Van Meerbeek *et al.*, 2003). Laboratory tests can evaluate the effect of a single variable, while keeping all other variables constant (Van Meerbeek *et al.*, 2003; De Munck *et al.*, 2005a).

In order to measure the bonding effectiveness of adhesives to enamel and dentin, diverse methodologies can today be used. The bond strength can be measured statically (macro-shear, macro-tensile, micro-shear, micro-tensile) or dynamically (fatigue test) (Van Meerbeek *et al.*, 2010).

Sano *et al.* (1994) developed the microtensile bond strength (μ TBS) test. This methodology has been accepted as a versatile and reliable *in vitro* static test to quantify the bonding effectiveness and stability of adhesive biomaterials bonded to tooth structure. It appears to be able to discriminate adhesives better on their bonding performance than a traditional shear bond strength approach (Armstrong *et al.*, 1998; Schreiner *et al.*, 1998; Pashley *et al.*, 1999; El Zohairy *et al.*, 2010; Poitevin *et al.*, 2010; Van Meerbeek *et al.*, 2010). Pashley *et al.* (1995) have stated a number of potential advantages for this methodology: more adhesive failures and fewer cohesive failures; higher interfacial bond strengths can be measured; the ability to measure regional bond strengths; means and variances can be calculated for single teeth; permits testing of bonds to irregular surfaces; permits testing of very small areas; and facilitates examination of the failed bonds by scanning electron microscopy, since the surface area is approximately 1 mm². Disadvantages of μ TBS test include: labor intensive and technically demanding; difficult to measure bond strengths <5 MPa, requires special equipment; samples are so small they dehydrate and damaged easily (Pashley *et al.*, 1995; Armstrong *et al.*, 1998; Pashley *et al.*, 1999).

Despite the importance of laboratory studies attempting to predict the clinical performance of biomaterials, clinical trials remain the ultimate way to collect scientific evidence on the clinical effectiveness of restorative treatment (Swift *et al.*, 1995; Van Meerbeek *et al.*, 2003; De Munck *et al.*, 2005a).

In spite of compromised *in vitro* and clinical longevity associated with ultrasimplified adhesives compared with that of adhesives that rely on several steps, some manufacturers have introduced recently more versatile adhesive systems, developed to simplify and shorten the application time, making the clinical procedure more user-friendly. These new adhesives can be applied either with the etch-and-rinse (two-step) or the etch-and-dry (one-step) technique. For etch-and-rinse adhesives, bond strength is derived both from resin tag and hybrid layer formation, with resin tags being responsible for an estimated one third of the bond strengths (Van Meerbeek *et al.*, 2003; De Munck *et al.*, 2005a). Otherwise, for etch-and-dry adhesives, chemical bonding has been shown to play a more important role. Mild self-etch adhesives rely on a combination of chemical adhesion to hydroxyapatite with some micromechanical interlocking that does not depend much on resin-tag formation (Van Meerbeek *et al.*, 2003; Yoshida *et al.*, 2004).

Hence, an adhesive that can be applied both ways and thus enables the practitioner to decide for a specific adhesive protocol, considered to be the most suited for the cavity prepared, would be very desirable. These new materials are called “**Universal**”, “**Multi-purpose**” or “**Multi-mode**” adhesives (Hanabusa *et al.*, 2012; Perdigao *et al.*, 2012; Yoshida *et al.*, 2012; Marchesi *et al.*, 2013; Mena-Serrano *et al.*, 2013; Munoz *et al.*, 2013; Perdigao *et al.*, 2013a; Perdigao *et al.*, 2013b; Taschner *et al.*, 2013; de Goes *et al.*, 2014; Munoz *et al.*, 2014; Perdigao & Loguercio, 2014; Pongprueksa *et al.*, 2014; Wagner *et al.*, 2014).

These new adhesives and the respective concept behind them are novel. Therefore, there is little information in the literature about the performance of this new class of adhesives. Only few ultramorphological and bond strength studies, and short-term clinical studies have been published. More *in vitro* and clinical studies are needed to back the use of these new materials.

For this reason, the purpose of the present work is to compare the immediate microtensile bond strengths (μ TBS) of one universal adhesive applied to dentin according to the etch-and-rinse and the self-etch strategies (Scotchbond Universal Adhesive, 3M ESPE, Neuss, Germany). The following null hypothesis was tested in this study: (1) There were no differences in dentin microtensile bond strength when the adhesive was applied either following an etch-and-rinse or etch-and-dry technique.

II. Materials and Methods

1. Type of study

Experimental *in vitro* study with the purpose of evaluating the microtensile bond strength of a universal adhesive system applied to dentin according to: 1) the etch-and-rinse strategy, or 2) the etch-and-dry strategy.

2. Design of the study

A convenient sample of six recently extracted human third molars, intact and without macroscopic evidence of caries or restorations, was used in this study. Before preparation, the teeth were randomly selected from a group of teeth, firstly stored in 0,5% Chloramine T (Sigma Chemical Co., St Louis, MO, USA) at 4°C for one week and then, left in distilled water at 4°C, according to the ISO TR 11405 standard, no more than three months. All teeth were cleaned under running water using a periodontal scaler before preparation.

3. Teeth preparation

From each tooth, a crown segment was obtained exposing middle dentin by sectioning the crowns with two cuts, a few millimeters apart, parallel to the occlusal surface, with a precision diamond disk at low speed (Diamond Wafering Blade - 10,2cmx0,3mm - Series 15HC, Buehler Ltd., Lake Bluff, IL, USA) (Figure 1), on a hard tissue microtome (Isomet 1000 Precision Saw, Buehler Ltd. Ltd., Lake Buff, IL, USA) (Figure 2) under constant distilled water irrigation, in the following way:

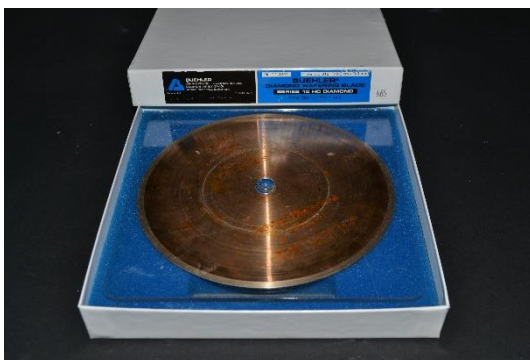


Figure 1 - Diamond wafering blade.



Figure 2 - Isomet 1000 Precision Saw, hard tissue microtome.

1. The teeth crowns were attached to an acrylic holder with sticky wax, perpendicular to the long axis of the tooth (Figure 3);



Figure 3 - Tooth attached to an acrylic holder with sticky wax.

2. The first cut was made parallel to the occlusal surface 1-2 mm below the cemento enamel junction to remove the roots (Figures 4 and 5);

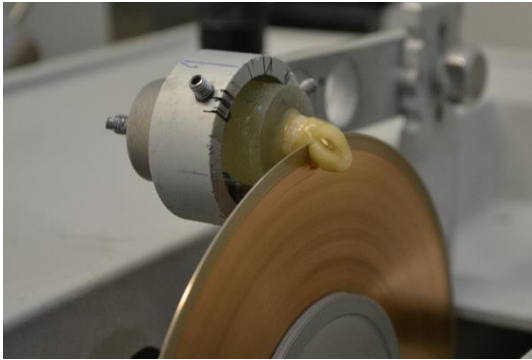


Figure 4 - Roots cutting 1-2 mm below CEJ.

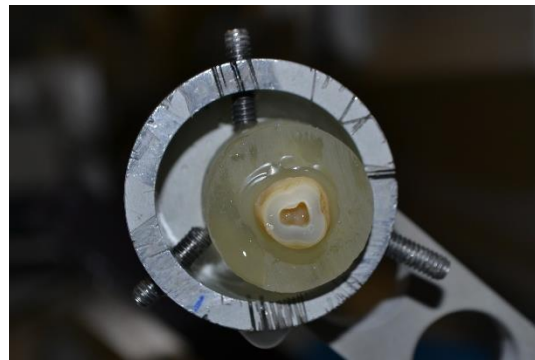


Figure 5 - Exposure of the pulp chamber after roots cut.

3. The crowns were detached from the acrylic holders and the pulp tissues were removed from the pulp chamber with a dentin curette (Figure 6). The pulp chamber was then filled with cyanoacrylate glue (737 Black Magic Toughened adhesive, Permabond, Hampshire, UK) (Figure 7) and the crowns were fixed with the same glue to the acrylic holders, by the sectioning surface (Figure 8).



Figure 6 - Removal of the pulp tissues.



Figure 7 - Filling of the pulp chamber with cyanoacrylate glue.



Figure 8 - The crown fixed to the acrylic holder.

4. Mid-coronal dentin surfaces were obtained by removing the occlusal enamel and superficial dentin of the molar crowns, perpendicular to the long axis of tooth (Figures 9 and 10).

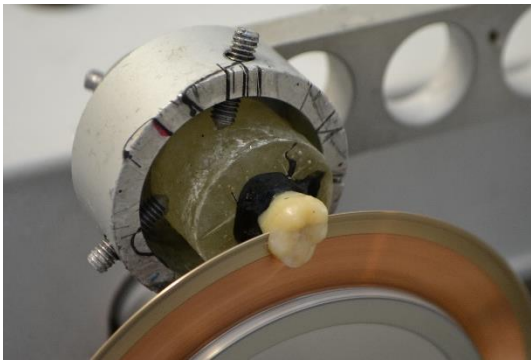


Figure 9 - Removal of the occlusal enamel and superficial dentin.



Figure 10 - Mid coronal dentin surface.

5. With the purpose of creating a uniform smear layer obtained in similar conditions to those occurring in clinic situations, the dentin surface was polished with 600-grit silica-carbide (SiC) sandpaper (Ultra-Prep, Buehler Ltd., Lake Bluff, IL, USA) on a mechanical grinder (Lunn Major, Struers, Denmark) (Figure 11), according to the ISO TR 11405 standard, during 60 seconds under water irrigation (Pashley *et al.*, 1988).



Figure 11 - Lunn Major, mechanical grinder.

4. Distribution and treatment of the crown segments

The six crown segments were randomly assigned to one of the two adhesive groups. The order in which the crown segments were treated was random, to avoid possible bias due to any particular sequence of treatment. All the treatment procedures were performed by the same operator in the way that is followed described:

Group A – Scotchbond Universal Adhesive (3M ESPE, Neuss, Germany) (Figure 12) as per manufacturer’s instructions – Self-etch (Etch-and-Dry) technique on dentin (SBU SE D):

Adhesive application

1. The occlusal surface was rinsed with water. The excess of water was removed from the dentin surface using a moist cotton pellet, so that the surface remained shiny and visibly moist.
2. The adhesive was applied, using a disposable microbrush, to the entire dentin surface, scrubbing lightly for 20 seconds.
3. The surface was then gently air-dried until it ceases to show any movement and the solvent was evaporated completely, forming a homogenous and slightly shiny film. Beginning with a soft blow of air from a distance of approximately 10 cm, the air pressure was increased while decreasing distance, finishing at a distance of approximately 1-2 mm from the surface at maximum air pressure.
4. Finally, the surface was polymerized for 10 seconds.

Group B – Scotchbond Universal Adhesive (3M ESPE, Neuss, Germany) as per manufacturer’s instructions – Total-etch (Etch-and-Rinse) technique on dentin (SBU TE D):

Conditioning step

1. The phosphoric acid etching gel was applied to the entire dentin surface, during 15 seconds. The occlusal surface was rinsed with water. The excess of water was removed from the dentin surface using a moist cotton pellet, so that the surface remained shiny and visibly moist.

Adhesive application

The adhesive was applied as for the self-etch mode.



Figure 12 - Scotchbond Universal Adhesive and Scotchbond Universal Etchant.

Resin composite build-ups were performed using a resin composite, ENAMEL Plus HRi (Micerium S.p.A. Avegno (GE) Italy) (Figures 13 and 14), shade UD4, in three increments of 2 mm each. Each increment was light cured for 20 seconds, according to the manufacturer's instructions, until reaching a height of 6 mm. Additional light polymerization was performed on facial, lingual, mesial and distal surfaces of the composite build-up for 10 seconds each.

All light curing was performed with a light intensity of 600 mW/cm² using a halogen light-activation unit (ELIPAR S10, 3M ESPE Seefeld, Germany), with the 13 mm light guide held 1-2 mm from the treatment surface. The output of the curing light was periodically verified at >600 mW/cm² with a radiometer (Curing Radiometer P/N 10503, USA) throughout the procedure.



Figure 13 - Resin composite ENAMEL Plus HRi.



Figure 14 - Resin composite build-up.

5. Specimens preparation for the microtensile tests

All teeth were painted with different colors with waterproof ink. The exterior surface of the resin composite was painted in order to identify, and then, exclude from the study, the sticks in which the adhesion was made to enamel.

The teeth were stored in distilled water in an incubator for 24 hours at 37 °C, according to the ISO TR 11405 standard. Date and time of the restoration was registered.

Posteriorly, the teeth were longitudinally sectioned in both “x” and “y” directions with a slow-speed diamond disk (Diamond Wafering Blade - 10,2cmx0,3mm - Series 15HC, Buehler Ltd., Lake Bluff, IL, USA) (Figure 15) under water irrigation, using a hard tissues microtome (Isomet Precision Saw, Buehler Ltd. Ltd., Lake Buff, IL, EUA), to obtain sticks with a cross-sectional area of approximately 0,7 mm².

A final cut is made at the base of the root, perpendicular to the long axis of the tooth, to separate the sticks from the acrylic supports (Figure 16).

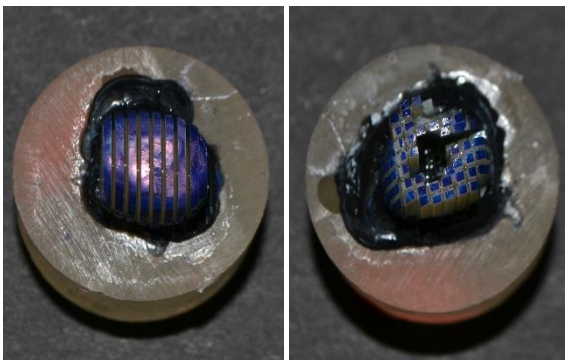


Figure 15 - The teeth after being sectioned in both “x” and “y” directions.



Figure 16 - The sticks obtained after the final cut.

Debonded or lost sticks are registered. Debonded sticks were those separated in the adhesive interface during the cutting procedure. Lost sticks were those which were lost or fractured during test preparation.

The obtained sticks were kept in distilled water for a maximum of 24 hours, until the conclusion of the microtensile tests.

6. Microtensile tests

The specimens were individually attached to a stainless-steel grooved Geraldelli's jig with cyanoacrylate glue (737 Black Magic Toughened adhesive, Permabond, Hampshire, UK) (Figure 17) and then submitted to a tension load using a universal testing machine (Instron 4502 Series, Serial no. H3307, Instron Corporation, Canton, MA, USA) (Figure 18), at a crosshead speed of 1mm/min until fracture occurred.

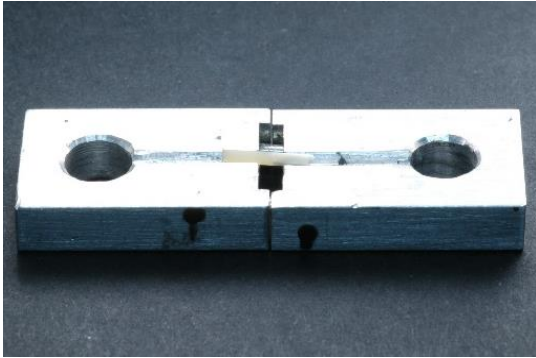


Figure 17 - The specimen attached to a Geraldelli's jig.



Figure 18 - Instron 4502, universal testing machine.

A digital caliper (Ficher Darex, France) was used to measure the sides of the bonding interface and calculate the bonding area in mm^2 . The load at fracture (KN) and the bonding surface area of the specimens were registered and μTBS calculated in MPa, by dividing the force imposed at time of fracture by the bonding area (mm^2).

The failure modes were analyzed under a stereomicroscope (Nikon, Japan) at 10X magnification and classified as: adhesive (A, failure occurring at the dentin-adhesive interface); cohesive in dentin (CD, when the failure occurred in dentin); cohesive in composite (CC, when the failure occurred in composite); and mixed (M, failure with composite and dentin at the interface).

7. Statistical Analysis

The statistical analysis of the results was performed through descriptive and inference methods. A paired-sample t-test was performed when the assumption of normality was valid: the Kolmogorov-Smirnov and Shapiro-Wilk Tests were used to assess whether the data followed a normal distribution (Table 1); the Levene's Tests was computed to determine if the assumption of equal variances was valid (Table 2). Significance was set at a 95% confidence level.

The number of prematurely debonded sticks (pretesting failures that occurred during specimen preparation) in each test group was recorded, but these values were not included in the statistical analysis (Perdigao *et al.*, 2006).

Group	Kolmogorov-Smirnov			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
<i>SBU TE D</i>	0,088	68	0,200	0,983	68	0,461
<i>SBU SE D</i>	0,084	72	0,200	0,981	72	0,351

Table 1 - Tests of Normality of the μ TBS in MPa for the two different experimental groups tested.

Levene's Test		
	F	Sig.
<i>Equal variances assumed</i>	3,334	0,070
<i>Equal variances not assumed</i>		

Table 2 - Test for Equality of Variances of the μ TBS in MPa.

III. Results

The number of microtensile sticks (N), mean and standard error (SE), standard deviation (SD), minimum (Min), maximum (Max) are shown in Table 3.

Group	N	Mean \pm SE	SD	Min	Max
SBU TE D	68	48,0 \pm 2,1	17,6	6,1	84,4
SBU SE D	72	56,9 \pm 2,5	21,2	7,2	99,6

Table 3 - Descriptive statistics of the μ TBS in MPa for the two experimental groups tested.

The highest mean μ TBS was obtained with SBU SE D (56,9 \pm 2,5 MPa) and the lowest was obtained with SBU TE D (48,0 \pm 2,1 MPa) (Figure 20).

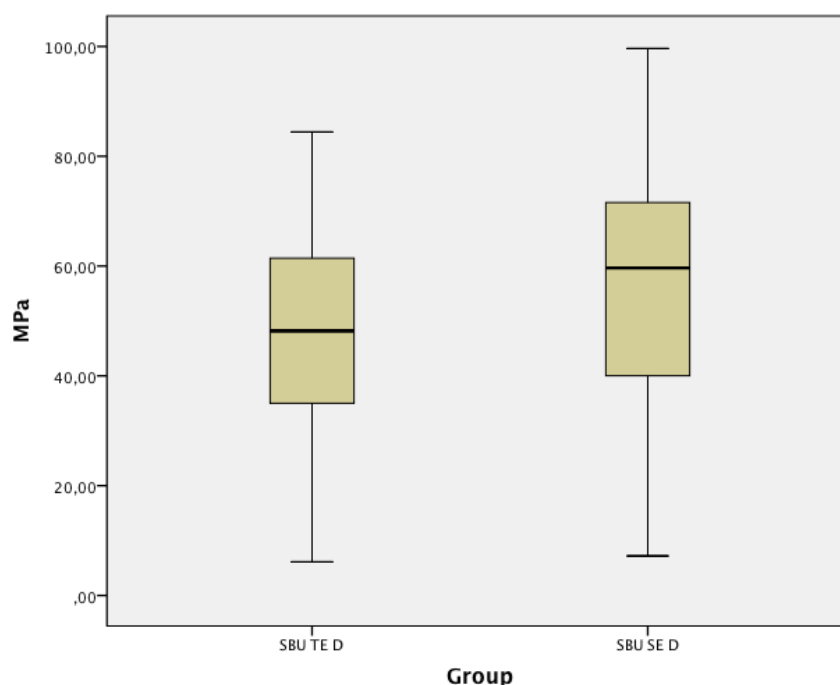


Figure 19 - Box-whisker plots of the μ TBS in MPa for the two different experimental groups tested. The median μ TBS is represented by the central line. The box represents the interquartile range.

After observing the normality of the data distribution and the equality of the variances, data from μ TBS were analyzed using a paired-sample t-test (Table 4).

t-test for Equality of Means				
	t	df	Sig. (2-tailed)	Mean Difference
Equal variances assumed	-2,673	138	0,008	-8,84586
Equal variances not assumed	-2,687	135,753	0,008	-8,84586

Table 4 - t-test for Equality of Means ($p \leq 0,05$).

The mean μ TBS to dentin of the Scotchbond Universal Adhesive applied following the etch-and-dry approach (SBU SE D) was statistically higher than that when the SBU was applied following the etch-and-rinse approach (SBU TE D) ($p = 0,008$).

Failure mode distribution of the debonded specimens is shown in Table 5 and 6, Figures 20 and 21. Fracture analysis revealed that the most predominant failure pattern was “adhesive” in both groups tested. Cohesive failures in composite and dentin were observed in 25% and 17,9% of the specimens, respectively. Mixed failures were observed in few specimens (7,8%).

Group	Mode of failure					
	N					
	Adhesive	Mixed	Cohesive in dentin	Cohesive in composite	Total	Pretesting failures
SBU TE D	40	10	8	10	68	4
SBU SE D	29	1	17	25	72	14

Table 5 - Number of specimens according to the failure mode and premature failures of the two different experimental groups tested.

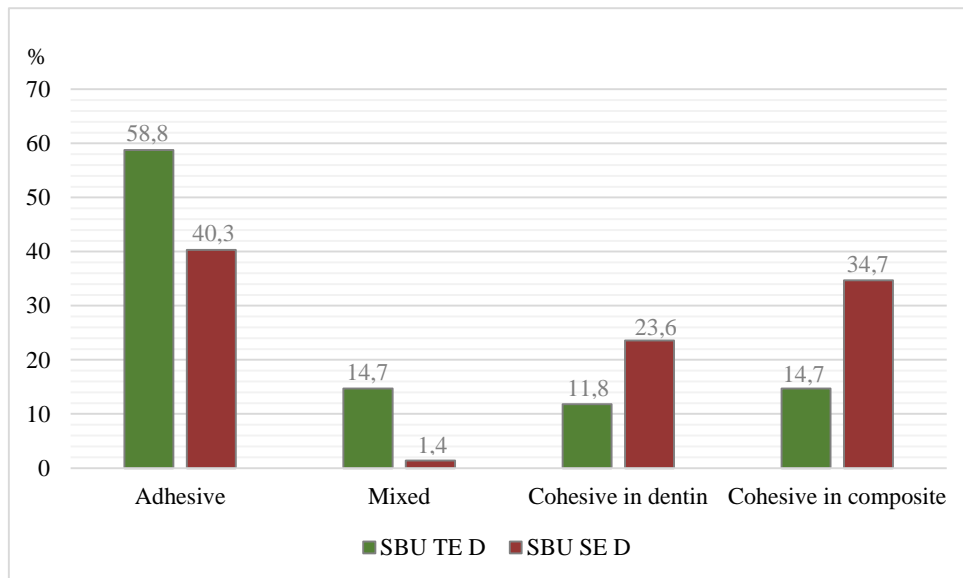


Figure 20 - Distribution of the specimens according to the failure mode of the two different experimental groups tested.

Mode of failure	N	%
<i>Adhesive</i>	69	49,3
<i>Mixed</i>	11	7,8
<i>Cohesive in dentin</i>	25	17,9
<i>Cohesive in composite</i>	35	25
	140	100

Table 6 - Number of specimens according to the failure mode and premature failures of all experimental groups tested.

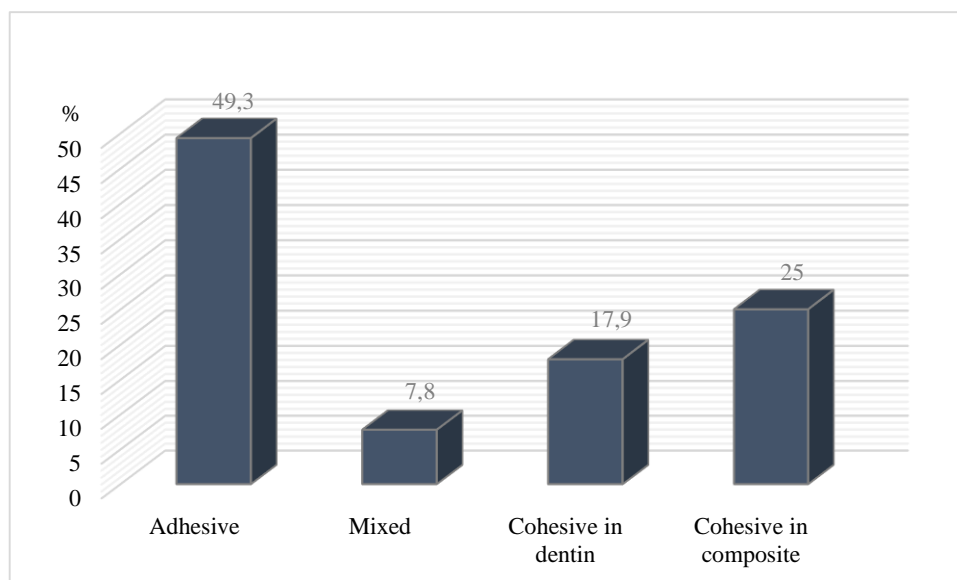


Figure 21 - Distribution of the specimens according to the failure mode of all experimental groups tested.

IV. Discussion

As the bond strength was measured upon 24 hours water storage, it should be considered as “immediate” bond strength. Universal adhesives represent the last generation of adhesives in the market. 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 the clinical situation, by application under different adhesive approach.

The null hypothesis was rejected, as there were statistical differences in dentin μ TBS among the different adhesion strategies for SBU: when SBU was applied following an etch-and-dry approach (SBU SE D) resulted in statistical higher μ TBS than when applied following an etch-and-rinse approach (SBU TE D) ($p=0,008$).

A possible reason for the higher performance of SBU when applied in the SE mode (SBU SE) is that the SBU bonding ability is two-fold, relying on a combination of chemical adhesion to hydroxyapatite (HAp) with some micromechanical interlocking that does not depend much on resin-tag formation (Van Meerbeek *et al.*, 2003; Yoshida *et al.*, 2004). In fact, the pH of Scotchbond Universal Adhesive is 2,7, which is within the range of the mild self-etch adhesives (Van Meerbeek *et al.*, 2003). Mild self-etch adhesives demineralise dentin only partially, keeping residual hydroxyapatite still attached to collagen. The abundant presence of HAp remaining around the collagen provides natural protection of the collagen, but also provides the potential for chemical interaction with HAp (Tay & Pashley, 2001; Van Meerbeek *et al.*, 2003; Yoshida *et al.*, 2004; De Munck *et al.*, 2005b). Molecules like 10-MDP and also polyalkenoic acid copolymer (PA) are present in SBU composition (Appendix 1) and will chemically bond to Ca of HAp, forming stable calcium-phosphate and calcium-carboxylate salts, respectively, along with only a limited surface-decalcification effect (Yoshida *et al.*, 2000; Fukuda *et al.*, 2003; Yoshida *et al.*, 2004; Van Meerbeek *et al.*, 2011; Perdigao *et al.*, 2012). Depending on the stability of the resulting calcium-monomer complex in adhesive suspension, this ionic bond may either decompose and demineralize the tooth surface, or remain stable and chemically bond with calcium (Yoshida *et al.*, 2000; Yoshida *et al.*, 2004). The monomer 10-MDP appears to not only interact more intensively with HAp, but also be hydrolytically more stable, as compared with other functional monomers as 4-MET and phenyl-P (Yoshida *et al.*, 2004; Van Landuyt *et al.*, 2007; Van Landuyt *et al.*, 2008). The high chemical affinity of 10-MDP for HAp was found to involve the formation of a self-assembled nano-layered structure of 10-MDP molecules, so-called “nanolayering”. Such nanolayering is typical of 10-MDP (could not be detected for the functional monomer 4-MET and phenyl-P) and may explain the high long-term stability of 10-MDP-based self-

etch adhesives (rather than its immediate bond strength), which has previously been proven both in laboratory and clinical research (Peumans *et al.*, 2010; Yoshihara *et al.*, 2010; Yoshihara *et al.*, 2011; Yoshida *et al.*, 2012). Beyond that, the partial dissolution of the dentin surface is sufficient to dissolve the smear layer and to provide some micro-mechanical interlocking. The thickness of the hybrid layer is, however, much smaller than that produced by the strong self-etch or etch-and-rinse adhesives, but has been proven to be minor in importance with regard to actual bonding effectiveness (Van Meerbeek *et al.*, 2003; De Munck *et al.*, 2005b; Van Meerbeek *et al.*, 2011).

The lower μ TBS obtained with SBU applied in the ER mode are in contrast with other studies of the same universal adhesive in the literature, reporting that phosphoric-acid etching of dentin prior to self-etch adhesive application (SBU TE) had no negative effect on microtensile bond strengths when compared to SBU SE (Perdigao *et al.*, 2012; Marchesi *et al.* 2013; Munoz *et al.*, 2013; Munoz *et al.*, 2014; Wagner *et al.*, 2014). It is known that smear layer constitutes a true physical barrier against the penetration of resin monomers, making the hybrid layer formation extremely difficult (Kenshima *et al.*, 2005; Kenshima *et al.*, 2006). After preliminary etching with phosphoric acid, the smear layer is removed and superficial dentin is demineralized. This seems to increase adhesive infiltration, allowing the creation of a well impregnated hybrid layer, when compared to their respective SE counterparts (Hanabusa *et al.*, 2012; Perdigao *et al.*, 2012; Taschner *et al.*, 2012; Munoz *et al.*, 2013; Wagner *et al.*, 2014). However, our results revealed that the group in which dentin was etched with phosphoric-acid prior to the application of a self-etch adhesive (SBU TE D) obtained inferior bond strengths, compared with its application on smear layer-covered dentin (SBU SE D). Phosphoric acid gels used with etch-and-rinse adhesives are more acidic than the phosphate or carboxylate monomers found in the composition of self-etch adhesives (Van Meerbeek *et al.*, 2003). Incomplete infiltration of the deeply demineralized collagen network by resin monomers was the main reason reported for this decrease in bond strength, due to the fact that phosphoric acid can decalcify human dentin more deeply (up to 3-6 μ m) than a (mild) self-etch adhesive is designed to infiltrate (Van Landuyt *et al.*, 2006a; Van Landuyt *et al.*, 2006b; Van Meerbeek *et al.*, 2011; Hanabusa *et al.*, 2012). In fact, the more resin that infiltrates acid-etched matrices, the higher are the resin-dentin bond strengths (Nishitani *et al.*, 2006). Such poorly resin-infiltrated zones and thus unprotected collagen fibrils are readily exposed to oral fluids. Hydrolytic breakdown, potentially accelerated by acids and enzymes produced by bacteria, and/or by enzymes present in dentin and activated by the

adhesive procedure, is expected to jeopardize the long-term stability of such porous etch-and-rinse hybrid layers (Breschi *et al.*, 2008; Pashley *et al.*, 2011). Previous studies in the literature (Van Landuyt *et al.*, 2006a; Van Landuyt *et al.*, 2006b), also reported a decreasing in the bond strength after phosphoric-acid etching of dentin prior to the application of a self-etch adhesive.

We could presume that the lower μ TBS obtained with SBU applied in the ER mode can also be attributed to the variability in the amount of moisture left by the operator in each dentin surface, before adhesive application. Over-drying and over-wetting are considered as two extremes (Tay *et al.*, 1998). In both situations will occur defects in the interface which could weak the resin-dentin adhesion: water excess leads to a decrease in adhesion as a result of the primer dilution and reduced conversion degree of the monomer (Jacobsen & Soderholm, 1995); over-drying dentin could rise the risk of collagen fibre collapse (Pashley *et al.*, 1992; Swift *et al.*, 1995). Between these two extremes is a “window of opportunity” wherein optimal hybridization and tubular seal may be achieved (Tay *et al.*, 1998). However, although most manufacturers of adhesive systems (including Scotchbond Universal Adhesive manufacturer) recommend the application of their materials to moist dentin and the adhesive application has been the same in all tested groups, it seems clear that the wet-bonding technique is difficult to standardize and sensitive to errors, since it is very difficult to determine the amount of water that should be kept on dentin surface before the adhesive application (Frankenberger *et al.*, 2000).

Since such adhesives combine the primer and the adhesive in the same solution, both hydrophilic and hydrophobic monomers are blended, with a relatively high concentration of solvent required to keep them in solution (Van Landuyt *et al.*, 2005). Consequently, increasing the risk of forming a hybrid layer without full involvement of the exposed collagen by adhesive monomers (Van Meerbeek *et al.*, 2001). This could be another possible explanation for the lower μ TBS obtained with SBU applied in the ER strategy.

In the present study, the most predominant failure pattern was adhesive in both SBU TE and SBU SE groups (58,8 and 40,3%, respectively) (figure 20). This is in accordance with previous studies in literature, reporting that more adhesive failures than cohesive failures are expected when performing a microtensile bond strength test (Pashley *et al.*, 1995; Schreiner *et al.*, 1998). Indeed, an accurate assessment of the strength of an adhesive material is best determined when the failure occurs within the

material itself and does not involve dentin or composite (Sano *et al.*, 1994; Ghassemieh, 2008).

Although the high number of adhesive failures obtained in this study, the number of cohesive failures in composite were higher than was expected for this kind of methodology (Pashley *et al.*, 1995; Schreiner *et al.*, 1998): in 25% of the specimens the fracture occurred exclusively in composite (Figure 21). Similar results were obtained previously suggesting that the curing time of the resin composite recommended by the manufacturer (20 seconds) was inferior to the ideal (Silva, 2008; Pequeno, 2009). In our study, the curing time of the resin composite recommended by the manufacturer was also 20 seconds. Proença *et al.* (2007) realized an *in vitro* study with ER and SE adhesives, however the resin composite was cured twice over (40 seconds) than the recommended time, and obtained less cohesive failures in composite. A previous study of Perdigao *et al.* (2006) obtained similar results for a curing time of 40 seconds. Although additional light polymerization was performed on facial, lingual, mesial and distal surfaces of the composite build-up for 10 seconds each, besides the 20 seconds recommended by the manufacturer, a high number of cohesive failures in composite were still observed. We could speculate that the additional light polymerization did not achieve the more central layers of the composite, taking into account that they had already been polymerized. Thus, we could presume that the additional light polymerization was useless in terms of preventing cohesive failures.

Cohesive failures in dentin were observed in 17,9% of the specimens (figure 21). Such cohesive failures in dentin do not mean that the resin-dentin bonds are uniformly stronger than the intrinsic strength of dentin, but that the manner in which the bond is stressed is so non-uniform that is concentrated or focused at one highly localized region where it opens a crack in dentin that then fails (Pashley *et al.*, 1995; Armstrong *et al.*, 1998; Pashley *et al.*, 1999). Moreover this type of failure is usually present when the sample has considerable damage or defect on the dentin side, which is hard to control (Phrukkanon *et al.*, 1998; Ghassemieh, 2008). For these reasons, this kind of failure pattern is somewhat expected and they should not be considered in the evaluation of the bond strengths (Ghassemieh, 2008).

Different types of imperfections and variations that might occur in the specimens can change the pattern of progression of failure, such as the misalignment of the axis of loading with specimen, the thickness of adhesive and uneven adhesion application (Ghassemieh, 2008). Thus, the specimens preparation for the microtensile tests requires

extremely careful manipulation (Cardoso *et al.*, 2001; De Munck *et al.*, 2005a), otherwise it may induce defects at the interface that may lead to lower bond strength values or even pre-testing failures (De Munck *et al.*, 2005a).

Pre-testing failures (ptf) were recorded, but not included in the statistical analysis. Correct interpretation of pre-testing failures with regard to the calculation of the average μ TBS is currently a matter of debate. There are studies that considered the ptf as (1) 0 MPa, which actually penalizes the adhesive too severely (as there was a certain bond strength), (2) others that excluded the ptf from the average μ TBS calculation and (3) others that a ptf received the lowest μ TBS measured within the respective group (Mine *et al.*, 2009). It is important to highlight that the non-inclusion of ptf in this study could have generated overestimated strength bond values (Wagner *et al.*, 2014), especially in the SE group, which obtained a high number of pre-testing failures, in spite of the higher μ TBS obtained. It is worth mentioning that these ptf occurred predominantly in only one tooth (Appendix 5), which was probably due to an error in adhesive application or the result of the entrapment of air bubbles in the adhesive layer.

The most used dentin preparation methods before bonding are preparation by either carbide or diamond bur or by silicon-carbide (SiC) paper (De Munck *et al.*, 2012). In this study, a thin artificial smear layer was created by means of grinding with 600-grit SiC paper, according to the ISO TR 11405 standard. Clinically, the smear layer created using a medium-grit diamond bur (average particle size of 70 μ m) is rougher than the smear layer produced by using 600-grit wet paper used in *in vitro* studies. Because increased smear-layer thickness and greater surface roughness correspond to lower SE adhesive impregnation, bond strength obtained after using a 600-grit paper (average particle size of 14,5 μ m) may be overestimated due to enhanced adhesive penetration (Oliveira *et al.*, 2003; Cardoso *et al.*, 2008; Taschner *et al.*, 2010), and thus, explaining the higher μ TBS obtained when SBU was applied in SE mode.

Taking into consideration that different bonding strategies (ER and SE) are influenced by chemical bonding and by micromechanical interlocking in different degrees, variation in regional characteristics of the substrate may also affects the two bonding strategies differently. However, there is some controversy as to whether sticks obtained from the peripheral area of the restored tooth result in decreased or increased μ TBS (Sezinando *et al.*, 2012). Whereas one study reported lower μ TBS for peripheral specimens than for centrally located specimens (Loguercio *et al.*, 2005), another study

reported that μ TBS to “periphery” dentin is higher than for the “central” specimens (De Munck *et al.*, 2011). In our study we used sticks from the entire interface, whereas Hanabusa (2012) only used nine central dentin sticks from each tooth to reduce substrate-regional variability. With this in mind, we should have divided the bonded sticks from each tooth in peripheral sticks and central sticks to avoid any bias in the μ TBS distribution caused by regional differences (Sarr *et al.*, 2010; Hanabusa *et al.*, 2012). In fact, water takes some time to diffuse from the external surface into the inner bonded interface, and thus, the resin-dentin interface at the periphery of the restoration (peripheral sticks) is more susceptible to water degradation than that located centrally (Loguercio *et al.*, 2005).

When performing microtensile tests, the stress is applied to a bonded surface area of 0,25-1 mm² (Sano *et al.*, 1994). The specimens used in this study had a cross-sectional area of approximately 0,7 mm², which corresponds to the bonding area size recommended by Phrukkanon and others (1998). An inverse relationship between dentin μ TBS and cross-sectional area was found (Sano *et al.*, 1994; Phrukkanon *et al.*, 1998). The reason for the increase in bond strength with decreases in bonded surface area is probably due to the presence of more defects (air bubbles, phase separations, surface roughness, nonuniform film thickness and angle of application of load) at the bonded interface or within the substrate of larger specimens. When the specimen is loaded, stress concentration occurs at the defects and initiates crack formation (Van Noort *et al.*, 1989; Sano *et al.*, 1994; Pashley *et al.*, 1995; Ghassemieh, 2008). The reduced bonding area of the sticks in this study could somehow explain the higher bond strengths obtained relatively to other studies, in the same conditions, but with sticks with a larger bonding area (Marchesi *et al.*, 2013; Munoz *et al.* 2013; Munoz *et al.*, 2014).

In spite of using a small bonding area, standard deviations were higher than expected, particularly for the SE group (figure 19). Apart from the bond strength, the variability is also an indicator for the performance of an adhesive. A low variability may indicate a good accuracy and low technique sensitivity of the adhesive. Variability in the bond strength results between different samples may be caused by several factors, such as the variability between teeth, variability in the application procedure, irregularities in the composite (such as air bubbles), the presence of voids in the adhesive (Phrukkanon *et al.*, 1998; Pashley *et al.*, 1999; Perdigao *et al.*, 2002). For these reasons, future studies should increase the number of teeth included in the sample, in an attempt to reduce the standard deviation.

It should be mentioned that we have detailed the manufacturer's instructions (Appendix 3). Due to commercial constraints, frequently manufacturers of adhesive systems when formulating the application instructions of their products, choose a solution between what would be the ideal instructions to maximize the results of a particular adhesive, and what would they think it would be acceptable for the majority of the clinicians. In fact, it was not possible to evaporate the solvent completely from the surface with a gentle stream of air over the liquid for only 5 seconds. Due to that reason the surface was gently air-dried until it ceases to show any movement and the solvent was evaporated completely, forming a homogenous and slightly shiny film. After the careful application of the primers, it is necessary to evaporate the solvent carefully. If this procedure is not respected, the solvents, which are used as transporters and facilitators of the resin infiltration, may prevent the formation of a perfectly infiltrated, sealed, fully polymerized hybrid layer (Pashley & Carvalho, 1997), and might result in accelerated bond degradation due to the lower polymerization conversion at the bonding interface (Ikeda *et al.*, 2008). Previous studies reported the importance of solvent/water evaporation with respect to dentin adhesion (Cavalheiro *et al.*, 2006; Cavalheiro, 2008).

One of the limitations of this study has to do with the lack of long-term water storage. With this in mind, future studies should analyze the effect of long-term water storage on the *in vitro* performance of the universal adhesive SBU.

The *in vitro* nature of this study does not allow direct extrapolation of the results to an *in vivo* situation, so whether the same results would be obtained *in vivo* should be the object of further investigation. Laboratory results do not always correlate with clinical performance. They should be regarded as screening tools, whose value lies only in their ability to discriminate between a multitude of adhesive systems and thus allowing researchers to focus on the more promising materials. Ultimately, the fact remains that the true performance of a material cannot be determined without rigorous clinical testing (El Zohairy *et al.*, 2010; Van Meerbeek *et al.*, 2010). It also should be made clear that tests used to evaluate bond strength of dental adhesives are not without their limitations. Another limitation of this study are that caries-free teeth were used. Specimens with any sign of caries were excluded because these lesions are seen as pathways for fluid escape and because there was a need for standardization. However, in a clinical scenario, the majority of the adhesive procedures are performed on teeth affected by caries lesions. Yet, it is known that caries significantly change the dentin permeability (Tagami *et al.*, 1992; Ceballos *et al.*, 2003; Scholtanus *et al.*, 2010).

V. Conclusions

Within the limitations of the present laboratory study, it may be concluded that improved bonding effectiveness of SBU on dentin is obtained when the adhesive is applied with the self-etch approach. The etch-and-rinse approach tested (wet dentin) resulted in immediate bond strength statistically lower than the self-etch group.

Due to the fact that these new adhesives and the respective concept behind them are novel, there is little information in the literature about the *in vitro* and *in vivo* performance of this new class of adhesives. Future studies should analyze the effect of long-term water storage on the *in vitro* performance of the universal adhesive SBU. Besides that, further studies on different substrates, such as carious dentin, in association with *in vivo* tests are needed to assess the long-term clinical behavior of this new class of adhesives and to back the use of these new materials.

Clinical significance: Improved bonding effectiveness of the tested universal adhesive system on dentin may be obtained if the adhesive is applied with the self-etch approach.

VI. Appendices

APPENDIX 1

Materials, Manufacturers, Components and Batch Numbers

Material	Manufacturer	Composition	Batch Number
<i>Scotchbond Universal Adhesive</i>	3M ESPE Neuss, Germany	Bis-GMA (15-25 vol%) HEMA (15-25 vol%) 10-MDP (5-15%) Ethanol (10-15 vol%) Water (10-15 vol%) Silane treated silica (5-15 vol%) 2-Propenoic acid, 2-Methyl-, reaction products with 1,10- Decanediol and phosphorous oxide (1-10 vol%) Copolymer of acrylic and itaconic acid (1-5 vol%) Dimethylaminobenzoat (<2 vol%) Camphorquinone (<2 vol%) (Dimethylamino)ethyl methacrylate (<2 vol%) Methyl ethyl ketone (<0,5 vol%)	Lot 540368 Exp 12/2015
<i>Scotchbond Universal Etchant</i>	3M ESPE Neuss, Germany	Water (50-65 vol%) Phosphoric acid (30-40 vol%) Synthetic amorphous silica, fumed, crystalline free (5-10 vol%) Polyethylene glycol (1-5 vol%) Aluminum oxide (<2 vol%)	Lot 537103 Exp 11/2015
<i>Enamel Plus HRi UD4</i>	Micerium S.P.A. Avegno, Italy	1,4-Butandioldimethacrylate Urethandimethacrylate Bis-GMA	Lot 2010006016 Exp 06/2015 Lot 2012000921 Exp 12/2018

Table 7 - Materials, Manufacturer, Composition and Batch Numbers.

APPENDIX 2

Application mode of Scotchbond Universal Adhesive according to the manufacturer's instructions

(information supplied in the safety data sheets and material instructions)

1) Tooth preparation

Remove loose preparation debris by spraying with water, and lightly air dry the cavity in only 2-3 bursts of water-free and oil-free air, or use cotton pellets to dry it off. Do not overdry.

The cavity should be just dry enough that the surface has a slightly glossy appearance. Overdrying can lead to post-operative sensitivity.

2) Procedure for direct restorations with light-curing composites

Adhesive System	Technique	Instructions for use
<i>Scotchbond Universal Adhesive</i>	<i>Etch-and-dry</i>	<ol style="list-style-type: none">1. Use the disposable applicator to apply the adhesive to the entire tooth structure and rub it in for 20 seconds;2. If necessary, rewet disposable applicator during treatment;3. Subsequently direct a gentle stream of air over the liquid for about 5 seconds until it no longer moves and the solvent has evaporated completely.4. Harden the adhesive with a commonly used curing light for 10 seconds.
	<i>Etch-and-rinse</i>	<ol style="list-style-type: none">1. Apply a commonly used phosphoric acid etching gel (about 35%), e.g., Scotchbond Universal Etchant, to the prepared and unprepared (if present) tooth structure (enamel and dentin) and allow to react for 15 seconds. Rinse thoroughly with water and dry with water-free and oil-free air or with cotton pellets. Do not overdry;2. Apply adhesive as for the self-etch mode.

Table 8 - Application mode of Scotchbond Universal Adhesive according to the manufacturer's instructions.

APPENDIX 3

Application mode of Scotchbond Universal Adhesive according to the detailed manufacturer's instructions

1) Occlusal surface preparation

The occlusal surface was rinsed with water. The excess of water was removed from the dentin surface using a moist cotton pellet, so that the surface remained shiny and visibly moist.

2) Procedure for direct restorations with light-curing composites

Adhesive System	Technique	Instructions for use
<i>Scotchbond Universal Adhesive</i>	<i>Etch-and-dry</i>	<ol style="list-style-type: none"> 1. The adhesive was applied, using a disposable microbrush, to the entire dentin surface, scrubbing lightly for 20 seconds. 2. The surface was then gently air-dried until it ceases to show any movement and the solvent was evaporated completely, forming a homogenous and slightly shiny film. Beginning with a soft blow of air from a distance of approximately 10 cm, the air pressure was increased while decreasing distance, finishing at a distance of approximately 1-2 mm from the surface at maximum air pressure. 3. Finally, the surface was polymerized for 10 seconds.
	<i>Etch-and-rinse</i>	<ol style="list-style-type: none"> 1. The phosphoric acid etching gel was applied to the entire dentin surface, during 15 seconds. The occlusal surface was rinsed with water. The excess of water was removed from the dentin surface using a moist cotton pellet, so that the surface remained shiny and visibly moist. 2. Apply adhesive as for the self-etch mode.

Table 9 - Application mode of Scotchbond Universal Adhesive according to the detailed manufacturer's instructions.

APPENDIX 4

Other materials



Figure 22 - Cyanoacrylate glue.



Figure 23 - Halogen light-activation unit.



Figure 24 - Incubator.

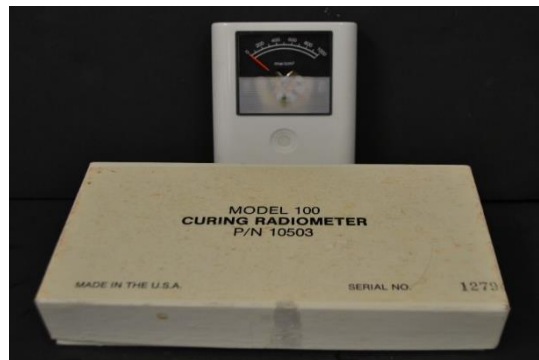


Figure 25 - Radiometer.



Figure 26 - Digital caliper.



Figure 27 - Stereomicroscope.

APPENDIX 5

Number of sticks obtained in each tooth

Group	Tooth	Sticks			
		<i>Lost</i>	<i>Pretesting failures</i>	Tested	Total
<i>SBU TE D</i>	<i>1</i>	2	3	22	27
	<i>2</i>	6	0	20	26
	<i>3</i>	2	1	26	29
<i>SBU SE D</i>	<i>1</i>	4	2	24	30
	<i>2</i>	1	3	29	33
	<i>3</i>	0	9	19	28

Table 10 - Number of sticks obtained in each tooth.

VII. References

Armstrong SR, Boyer DB, Keller JC. Microtensile bond strength testing and failure analysis of two dentin adhesives. *Dental materials : official publication of the Academy of Dental Materials* 1998; 14(1):44-50.

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. *Dental materials : official publication of the Academy of Dental Materials* 2008; 24(1):90-101.

Cardoso MV, Coutinho E, Ermis RB, Poitevin A, Van Landuyt K, De Munck J, et al. Influence of dentin cavity surface finishing on micro-tensile bond strength of adhesives. *Dental materials : official publication of the Academy of Dental Materials* 2008; 24(4):492-501.

Cardoso PE, Carrilho MR, Francci CE, Perdigao J. Microtensile bond strengths of one-bottle dentin adhesives. *American journal of dentistry* 2001; 14(1):22-4.

Cavalheiro A, Vargas MA, Armstrong SR, Dawson DV, Gratton DG. Effect of incorrect primer application on dentin permeability. *The journal of adhesive dentistry* 2006; 8(6):393-400.

Cavalheiro A. Effect of deviations on the application of dentin adhesives: permeability, ultramorphology and nanoleakage studies (dissertation). Lisbon: School of Dentistry, Univ. of Lisbon; 2008.

Ceballos L, Camejo DG, Victoria Fuentes M, Osorio R, Toledano M, Carvalho RM, et al. Microtensile bond strength of total-etch and self-etching adhesives to caries-affected dentine. *Journal of dentistry* 2003; 31(7):469-77.

de Goes MF, Shinohara MS, Freitas MS. Performance of a New One-step Multi-mode Adhesive on Etched vs Non-etched Enamel on Bond Strength and Interfacial Morphology. *The journal of adhesive dentistry* 2014.

De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Vargas M, Suzuki K, et al. Four-year water degradation of total-etch adhesives bonded to dentin. *Journal of dental research* 2003; 82(2):136-40.

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. *Journal of dental research* 2005a; 84(2):118-32.

De Munck J, Vargas M, Iracki J, Van Landuyt K, Poitevin A, Lambrechts P, et al. One-day bonding effectiveness of new self-etch adhesives to bur-cut enamel and dentin. *Operative dentistry* 2005b; 30(1):39-49.

De Munck J, Van den Steen PE, Mine A, Van Landuyt KL, Poitevin A, Opdenakker G, et al. Inhibition of enzymatic degradation of adhesive-dentin interfaces. *Journal of dental research* 2009; 88(12):1101-6.

De Munck J, Mine A, Vivian Cardoso M, De Almeida Neves A, Van Landuyt KL, Poitevin A, et al. Effect of dentin location and long-term water storage on bonding effectiveness of dentin adhesives. *Dental materials journal* 2011; 30(1):7-13.

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. *Journal of dental research* 2012; 91(4):351-7.

El Zohairy AA, Saber MH, Abdalla AI, Feilzer AJ. Efficacy of microtensile versus microshear bond testing for evaluation of bond strength of dental adhesive systems to enamel. *Dental materials : official publication of the Academy of Dental Materials* 2010; 26(9):848-54.

Frankenberger R, Kramer N, Petschelt A. Technique sensitivity of dentin bonding: effect of application mistakes on bond strength and marginal adaptation. *Operative dentistry* 2000; 25(4):324-30.

Frankenberger R, Perdigao J, Rosa BT, Lopes M. "No-bottle" vs "multi-bottle" dentin adhesives--a microtensile bond strength and morphological study. *Dental materials : official publication of the Academy of Dental Materials* 2001; 17(5):373-80.

Fukuda R, Yoshida Y, Nakayama Y, Okazaki M, Inoue S, Sano H, et al. Bonding efficacy of polyalkenoic acids to hydroxyapatite, enamel and dentin. *Biomaterials* 2003; 24(11):1861-7.

Ghassemieh E. Evaluation of sources of uncertainties in microtensile bond strength of dental adhesive system for different specimen geometries. *Dental materials : official publication of the Academy of Dental Materials* 2008; 24(4):536-47.

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. *Journal of dentistry* 2012; 40(6):475-84.

Hashimoto M, Ohno H, Endo K, Kaga M, Sano H, Oguchi H. The effect of hybrid layer thickness on bond strength: demineralized dentin zone of the hybrid layer. *Dental materials : official publication of the Academy of Dental Materials* 2000; 16(6):406-11.

Ikeda T, De Munck J, Shirai K, Hikita K, Inoue S, Sano H, et al. Effect of air-drying and solvent evaporation on the strength of HEMA-rich versus HEMA-free one-step adhesives. *Dental materials : official publication of the Academy of Dental Materials* 2008; 24(10):1316-23.

International Standardization Organization (2003). Technical specification ISO/TS 11405. *Dental materials – Testing of adhesion to tooth structure: 1-6.*

Jacobsen T, Soderholm KJ. Some effects of water on dentin bonding. *Dental materials : official publication of the Academy of Dental Materials* 1995; 11(2):132-6.

Kenshima S, Reis A, Uceda-Gomez N, Tancredo Lde L, Filho LE, Nogueira FN, et al. Effect of smear layer thickness and pH of self-etching adhesive systems on the bond strength and gap formation to dentin. *The journal of adhesive dentistry* 2005; 7(2):117-26.

Kenshima S, Francci C, Reis A, Loguercio AD, Filho LE. Conditioning effect on dentin, resin tags and hybrid layer of different acidity self-etch adhesives applied to thick and thin smear layer. *Journal of dentistry* 2006; 34(10):775-83.

Loguercio AD, Uceda-Gomez N, Carrilho MR, Reis A. Influence of specimen size and regional variation on long-term resin-dentin bond strength. *Dental materials : official publication of the Academy of Dental Materials* 2005; 21(3):224-31.

Marchesi G, Frassetto A, Mazzoni A, Apolonio F, Diolosa M, Cadenaro M, et al. Adhesive performance of a multi-mode adhesive system: 1-Year *in vitro* study. *Journal of dentistry* 2013.

Mazzoni A, Scaffa P, Carrilho M, Tjaderhane L, Di Lenarda R, Polimeni A, et al. Effects of etch-and-rinse and self-etch adhesives on dentin MMP-2 and MMP-9. *Journal of dental research* 2013; 92(1):82-6.

Mena-Serrano A, Kose C, De Paula EA, Tay LY, Reis A, Loguercio AD, et al. A new universal simplified adhesive: 6-month clinical evaluation. *Journal of esthetic and restorative dentistry : official publication of the American Academy of Esthetic Dentistry [et al]* 2013; 25(1):55-69.

Mine A, De Munck J, Cardoso MV, Van Landuyt KL, Poitevin A, Kuboki T, et al. Bonding effectiveness of two contemporary self-etch adhesives to enamel and dentin. *Journal of dentistry* 2009; 37(11):872-83.

Munoz MA, Luque I, Hass V, Reis A, Loguercio AD, Bombarda NH. Immediate bonding properties of universal adhesives to dentine. *Journal of dentistry* 2013; 41(5):404-11.

Munoz MA, Sezinando A, Luque-Martinez I, Szesz AL, Reis A, Loguercio AD, et al. Influence of a hydrophobic resin coating on the bonding efficacy of three universal adhesives. *Journal of dentistry* 2014.

Nakabayashi N, Kojima K, Masuhara E. The promotion of adhesion by the infiltration of monomers into tooth substrates. *Journal of biomedical materials research* 1982; 16(3):265-73.

Nishitani Y, Yoshiyama M, Donnelly AM, Agee KA, Sword J, Tay FR, et al. Effects of resin hydrophilicity on dentin bond strength. *Journal of dental research* 2006; 85(11):1016-21.

Nunes MF, Swift EJ, Jr., Perdigao J. Effects of demineralization depth on microtensile bond strength to human dentin. *The journal of adhesive dentistry* 2001; 3(2):137-43.

Oliveira SS, Pugach MK, Hilton JF, Watanabe LG, Marshall SJ, Marshall GW, Jr. The influence of the dentin smear layer on adhesion: a self-etching primer vs. a total-etch

system. Dental materials : official publication of the Academy of Dental Materials 2003; 19(8):758-67.

Pashley DH, Derkson GD, Tao L, Derkson M, Kalathoor S. The effects of a multi-step dentin bonding system on dentin permeability. Dental materials : official publication of the Academy of Dental Materials 1988; 4(2):60-3.

Pashley DH, Horner JA, Brewer PD. Interactions of conditioners on the dentin surface. Operative dentistry 1992; Suppl 5:137-50.

Pashley DH, Sano H, Ciucchi B, Yoshiyama M, Carvalho RM. Adhesion testing of dentin bonding agents: a review. Dental materials : official publication of the Academy of Dental Materials 1995; 11(2):117-25.

Pashley DH, Carvalho RM. Dentine permeability and dentine adhesion. Journal of dentistry 1997; 25(5):355-72.

Pashley DH, Carvalho RM, Sano H, Nakajima M, Yoshiyama M, Shono Y, et al. The microtensile bond test: a review. The journal of adhesive dentistry 1999; 1(4):299-309.

Pashley DH, Tay FR. Aggressiveness of contemporary self-etching adhesives. Part II: etching effects on unground enamel. Dental materials : official publication of the Academy of Dental Materials 2001; 17(5):430-44.

Pashley DH, Tay FR, Breschi L, Tjaderhane L, Carvalho RM, Carrilho M, et al. State of the art etch-and-rinse adhesives. Dental materials : official publication of the Academy of Dental Materials 2011; 27(1):1-16.

Pequeno A. Efeito da alteração do modo de aplicação de um adesivo *etch-and-rinse* nas forças de adesão (dissertação). Lisboa: Instituto Superior de Ciências da Saúde Egas Moniz; 2009.

Perdigao J, Frankenberger R, Rosa BT, Breschi L. New trends in dentin/enamel adhesion. American journal of dentistry 2000; 13(Spec No):25d-30d.

Perdigao J, Lopes M. The effect of etching time on dentin demineralization. Quintessence Int 2001; 32(1):19-26.

Perdigao J, Geraldeli S, Carmo AR, Dutra HR. *In vivo* influence of residual moisture on microtensile bond strengths of one-bottle adhesives. *Journal of esthetic and restorative dentistry : official publication of the American Academy of Esthetic Dentistry [et al]* 2002; 14(1):31-8.

Perdigao J, Gomes G, Gondo R, Fundingsland JW. *In vitro* bonding performance of all-in-one adhesives. Part I--microtensile bond strengths. *The journal of adhesive dentistry* 2006; 8(6):367-73.

Perdigao J, Sezinando A, Monteiro PC. Laboratory bonding ability of a multi-purpose dentin adhesive. *American journal of dentistry* 2012; 25(3):153-8.

Perdigao J, Kose C, Mena-Serrano A, De Paula E, Tay L, Reis A, et al. A New Universal Simplified Adhesive: 18-Month Clinical Evaluation. *Operative dentistry* 2013a.

Perdigao J, Munoz M, Sezinando A, Luque-Martinez I, Staichak R, Reis A, et al. Immediate Adhesive Properties to Dentin and Enamel of a Universal Adhesive Associated With a Hydrophobic Resin Coat. *Operative dentistry* 2013b.

Perdigao J, Loguercio AD. Universal or Multi-mode Adhesives: Why and How? *The journal of adhesive dentistry* 2014; 16(2):193-4.

Peumans M, De Munck J, Van Landuyt KL, Poitevin A, Lambrechts P, Van Meerbeek B. Eight-year clinical evaluation of a 2-step self-etch adhesive with and without selective enamel etching. *Dental materials : official publication of the Academy of Dental Materials* 2010; 26(12):1176-84.

Phrukkanon S, Burrow MF, Tyas MJ. Effect of cross-sectional surface area on bond strengths between resin and dentin. *Dental materials : official publication of the Academy of Dental Materials* 1998; 14(2):120-8.

Poitevin A, De Munck J, Cardoso MV, Mine A, Peumans M, Lambrechts P, et al. Dynamic versus static bond-strength testing of adhesive interfaces. *Dental materials : official publication of the Academy of Dental Materials* 2010; 26(11):1068-76.

Pongprueksa P, Miletic V, De Munck J, Brooks N, Meersman F, Nies E, et al. Effect of Evaporation on the Shelf Life of a Universal Adhesive. *Operative dentistry* 2014.

Proenca JP, Polido M, Osorio E, Erhardt MC, Aguilera FS, Garcia-Godoy F, et al. Dentin regional bond strength of self-etch and total-etch adhesive systems. *Dental materials : official publication of the Academy of Dental Materials* 2007; 23(12):1542-8.

Sano H, Shono T, Sonoda H, Takatsu T, Ciucchi B, Carvalho R, et al. Relationship between surface area for adhesion and tensile bond strength--evaluation of a micro-tensile bond test. *Dental materials : official publication of the Academy of Dental Materials* 1994; 10(4):236-40.

Sarr M, Kane AW, Vreven J, Mine A, Van Landuyt KL, Peumans M, et al. Microtensile bond strength and interfacial characterization of 11 contemporary adhesives bonded to bur-cut dentin. *Operative dentistry* 2010; 35(1):94-104.

Scholtanus JD, Purwanta K, Dogan N, Kleverlaan CJ, Feilzer AJ. Microtensile bond strength of three simplified adhesive systems to caries-affected dentin. *The journal of adhesive dentistry* 2010; 12(4):273-8.

Schreiner RF, Chappell RP, Glaros AG, Eick JD. Microtensile testing of dentin adhesives. *Dental materials : official publication of the Academy of Dental Materials* 1998; 14(3):194-201.

Sezinando A, Perdigao J, Regalheiro R. Dentin bond strengths of four adhesion strategies after thermal fatigue and 6-month water storage. *Journal of esthetic and restorative dentistry : official publication of the American Academy of Esthetic Dentistry [et al]* 2012; 24(5):345-55.

Silva A. Efeito do tempo de polimerização nas forças de adesão entre a dentina e as resinas compostas (dissertação). Lisboa: Instituto Superior de Ciências da Saúde Egas Moniz; 2008.

Swift EJ, Jr., Perdigao J, Heymann HO. Bonding to enamel and dentin: a brief history and state of the art, 1995. *Quintessence Int* 1995; 26(2):95-110.

Tagami J, Hosoda H, Burrow MF, Nakajima M. Effect of aging and caries on dentin permeability. *Proceedings of the Finnish Dental Society Suomen Hammaslaakariseuran toimituksia* 1992; 88 Suppl 1:149-54.

Taschner M, Nato F, Mazzoni A, Frankenberger R, Kramer N, Di Lenarda R, et al. Role of preliminary etching for one-step self-etch adhesives. *European journal of oral sciences* 2010; 118(5):517-24.

Taschner M, Nato F, Mazzoni A, Frankenberger R, Falconi M, Petschelt A, et al. Influence of preliminary etching on the stability of bonds created by one-step self-etch bonding systems. *European journal of oral sciences* 2012; 120(3):239-48.

Taschner M, Kummerling M, Lohbauer U, Breschi L, Petschelt A, Frankenberger R. Effect of Double-layer Application on Dentin Bond Durability of One-step Self-etch Adhesives. *Operative dentistry* 2013.

Tay FR, Gwinnett JA, Wei SH. Relation between water content in acetone/alcohol-based primer and interfacial ultrastructure. *Journal of dentistry* 1998; 26(2):147-56.

Tay FR, Pashley DH. Aggressiveness of contemporary self-etching systems. I: Depth of penetration beyond dentin smear layers. *Dental materials : official publication of the Academy of Dental Materials* 2001; 17(4):296-308.

Van Landuyt KL, De Munck J, Snauwaert J, Coutinho E, Poitevin A, Yoshida Y, et al. Monomer-solvent phase separation in one-step self-etch adhesives. *Journal of dental research* 2005; 84(2):183-8.

Van Landuyt KL, Kanumilli P, De Munck J, Peumans M, Lambrechts P, Van Meerbeek B. Bond strength of a mild self-etch adhesive with and without prior acid-etching. *Journal of dentistry* 2006a; 34(1):77-85.

Van Landuyt KL, Peumans M, De Munck J, Lambrechts P, Van Meerbeek B. Extension of a one-step self-etch adhesive into a multi-step adhesive. *Dental materials : official publication of the Academy of Dental Materials* 2006b; 22(6):533-44.

Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, et al. Systematic review of the chemical composition of contemporary dental adhesives. *Biomaterials* 2007; 28(26):3757-85.

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. *Journal of dental research* 2008; 87(8):757-61.

Van Meerbeek B, Perdigao J, Lambrechts P, Vanherle G. The clinical performance of adhesives. *Journal of dentistry* 1998; 26(1):1-20.

Van Meerbeek B, Vargas M, Inoue S, Yoshida Y, Peumans M, Lambrechts P. Adhesives and cements to promote preservation dentistry. *Operative dentistry* 2001; 6:119-44.

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. *Operative dentistry* 2003; 28(3):215-35.

Van Meerbeek B, Peumans M, Poitevin A, Mine A, Van Ende A, Neves A, et al. Relationship between bond-strength tests and clinical outcomes. *Dental materials : official publication of the Academy of Dental Materials* 2010; 26(2):e100-21.

Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt KL. State of the art of self-etch adhesives. *Dental materials : official publication of the Academy of Dental Materials* 2011; 27(1):17-28.

Van Noort R, Noroozi S, Howard IC, Cardew G. A critique of bond strength measurements. *Journal of dentistry* 1989; 17(2):61-7.

Wagner A, Wendler M, Petschelt A, Belli R, Lohbauer U. Bonding performance of universal adhesives in different etching modes. *Journal of dentistry* 2014.

Yoshida Y, Van Meerbeek B, Nakayama Y, Snauwaert J, Hellemans L, Lambrechts P, et al. Evidence of chemical bonding at biomaterial-hard tissue interfaces. *Journal of dental research* 2000; 79(2):709-14.

Yoshida Y, Nagakane K, Fukuda R, Nakayama Y, Okazaki M, Shintani H, et al. Comparative study on adhesive performance of functional monomers. *Journal of dental research* 2004; 83(6):454-8.

Yoshida Y, Yoshihara K, Nagaoka N, Hayakawa S, Torii Y, Ogawa T, et al. Self-assembled Nano-layering at the Adhesive interface. *Journal of dental research* 2012; 91(4):376-81.

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 biomaterialia* 2010; 6(9):3573-82.

Yoshihara K, Yoshida Y, Hayakawa S, Nagaoka N, Irie M, Ogawa T, et al. Nanolayering of phosphoric acid ester monomer on enamel and dentin. *Acta biomaterialia* 2011; 7(8):3187-95.