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Microtensile dentin bond strength evaluation of different adhesive systems: *in vitro* study

Sandra Filipa Seabra de Campos

Orientador: Prof. Doutor João Carlos Ramos

Coorientador: Dr. Fernando Marques

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Microtensile dentin bond strength evaluation of different adhesive systems: *in vitro* study

Campos, S.; Marques, F.; Ramos, J. C.

Área de Medicina Dentária, Faculdade de Medicina, Universidade de Coimbra
Av. Bissaya Barreto, Blocos de Celas
300-075 Coimbra
Portugal

E-mail: sfsdcampos@gmail.com

Abstract

Introduction: Modern restorative dentistry has been extensively influenced by the fast progress in dental adhesive technology. To assess the effectiveness of adhesive systems and to predict their clinical performance the resin-dentin bond strength tests has been widely used.

Purpose: The goal of this study was to compare the microtensile bond strength of five different adhesives systems at the dentin-composite interface and evaluate failure modes.

Materials and methods: Flat dentin surfaces were prepared in 25 non-carious human molars. Exposed dentin surfaces were wet-grounded with 240-, 400- and 600-grit silicon-carbide sandpaper to create bonding surfaces with a standardized smear layer. The teeth were randomly divided into 5 distinct groups according to the adhesive system tested: three self-etching adhesive systems, Xeno® V+ (Dentsply DeTrey, Konstanz, Germany); Xeno® III (Dentsply DeTrey, Konstanz, Germany) and Clearfil™ SE Bond (Kuraray Medical Inc., Okayama, Japan), and two etch-and-rinse adhesive systems, OptiBond™ FL (Kerr, Orange, CA, USA) and Prime&Bond® NT (Dentsply DeTrey, Konstanz, Germany) applied with respect to manufacturer's instructions. After adhesive procedures a 4-mm thick composite crown was built over the bonded surface. Following the storage in distilled water at 37 ºC, the samples were vertically cross-sectioned until obtaining sticks with 1.37mm^2 of cross-sectional area which were tested in tension in a universal testing machine at 0.5 mm/min. Data were analyzed by one-away ANOVA and a Tukey HSD post-hoc test ($p<0.05$). The mode of failure was also analysed with optical microscopy. Additionally, dentin disks were obtained, treated with the different conditioners and primers and observed by scanning electron microscopy (SEM).

Results: The following microtensile bond strengths were registered (mean in MPa±SD): Group I – Xeno® V+ 3.70±5.01; Group II - Xeno® III 18.94±13.87; Group III – OptiBond™ FL 43.29±12.74; Group IV- Prime&Bond® NT 39.64±15.06 and Group V – Clearfil™ SE Bond 42.80±10.65. Etch-and-rinse (OptiBond™ FL; Prime & Bond® NT) and two-step self-etching (Clearfil™ SE Bond) adhesive systems attained significant higher microtensile bond strength, without statistically significant differences between them. Significant lower values were obtained for Xeno® V+ comparing to Xeno® III. Cohesive composite failures were related with the higher bond strength values, whereas the adhesive failures were associated with the lowest bond strength values.

Conclusion: Among the materials evaluated, etch-and-rinse and two-step self-etching adhesive systems presented higher dentin bond strength than the one-step self-etching adhesive systems.

Keywords: microtensile; dentin bond strength; etch-and-rinse; self-etch; dentin adhesion

Introduction

Improvements in dental adhesive technology have extensively influenced modern restorative dentistry[1]. Today's, operative dentistry should primary involve "minimally invasive" techniques[1, 2] that promotes a more conservative cavity design, which basically relies on the effectiveness of current enamel-dentine adhesives[1].

Bonding to enamel has been demonstrated to be easy and durable while bonding to dentin is far more challenging[3], due to the heterogeneity of structure and composition of dentin, its surface characteristics after bur cutting and chemical treatments[1, 3-7] and relation with pulpal tissue by means of numerous fluid-filled tubules[1]. Basically, current dentin adhesives employ two different means to achieve the goal of retention between restorative material and dentin[8]: by removing the smear layer with etch-and-rinse adhesives or modifying the smear layer with self-etch adhesives.

The adhesion to tooth substrate implies an exchange process in which some inorganic tooth material is removed and replaced by resin monomers[2, 9] that, upon polymerization, become micromechanically interlocked in the created microporosities[10, 11] , rather than on a primary chemical adhesion[12]. However, this twofold bonding mechanism is believed to be advantageous in terms of restoration longevity[13,14].

Contemporary dental adhesive systems can be classified according to the application techniques as etch-and-rinse and self-etching adhesives systems[15]. In the first one, the acid-etching application completely removes the smear layer, followed by the application of primer and bond resin in one or two steps[16]. The etch-and-rinse technique is still the most effective and stable approach for enamel bonding[2]. However, concerning dentin bonding, this technique can be considered to be difficult and less predictable[1]. With the self-etching adhesive systems the clinician can simplify the clinical procedures and also reducing clinical time[17], therefore to reduce technique sensitivity or risk of making errors during application and manipulation[2]. Nevertheless, some potential problems are related with the use of some self-etching adhesives, like postoperative sensivity, incomplete marginal seal, premature bond degradation, biocompatibility, and compromised bonding to abnormal substrates[18].

The self-etch technique does not require a separating etching step, it uses acidic monomers that simultaneously etch and prime the dental substrate[1]. The self-etch adhesive systems can be classified according to the pH of the adhesive solutions in strong ($pH<1$), intermediately strong or moderate (pH between 1.0 and 2.0) and mild ($pH>2$)[2]. The high acidity results in rather deep demineralization effects. So, the bonding mechanism of "strong" self-etching adhesives can be similar to the etch-and-rinse approach[2]. However, low-pH self-etch adhesives reveals low bond strength values, especially at dentin[2] with a high number of pre-testing failures, especially when tested with a microtensile methodology[2].

The aim of this study was to compare the dentin microtensile bond strength of five adhesive systems.

The null hypothesis was that there were no significant differences between the five adhesives systems evaluated.

Materials and methods

Specimen preparation

Twenty-five non-carious human molars were collected and stored in distilled water within 10 weeks after extraction. The teeth were cleaned from debris and partially included in an acrylic resin block (Orthocryl®, Dentaurum). The oclusal 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 oclusal surfaces were wet-ground with a sequence of 240-, 400- and 600-grit silicon-carbide sandpaper in circular motion for 60 seconds to obtain a uniform smear layer. The prepared oclusal surfaces were carefully observed using an optical microscope at a 40-fold magnification (M300, Leica, Switzerland) to confirm the absence of residual enamel or another defects in dentin surfaces.

Bonding and restorative procedures

The teeth were randomly divided in five groups, according the five adhesive systems tested: two and three-step etch-and-rinse adhesives and one and two-step self-etch adhesives (Table I).

Table I: Adhesive systems studied, manufacturers, chemical composition, pH values and batch numbers.

Adhesive	Manufacturer	Chemical Composition	pH	Batch no.
Group I Xeno® V+ 1-step/ 1 bottle Self-etch Adhesive	Dentsply DeTrey, Konstanz, Germany	-Bifunctional acrylate -Acidic acrylate - Functionalized phosphoric acid ester -Water -Tertiary butanol -Initiator - Stabilizer	1.3[19]	1203000016
Group II Xeno® III 1-step/ 2 bottles Self-etch Adhesive	Dentsply DeTrey, Konstanz, Germany	Liquid A: -HEMA; purified water; ethanol; BHT; highly disperse silicone dioxide Liquid B: -Pyro-EMA; PEM-F; urethane dimethacrylate; BHT; camphorquinone; ethyl-4-dimethylaminobenzoate	1.4[2]	1302000019
Group III OptiBond™ FL 3-step Etch-and-rinse adhesive	Kerr, Orange, CA, USA	Etchant: 37.5% phosphoric acid Primer: -HEMA;GPDM; PAMM; ethanol; water; photo initiator Adhesive: - TEGDMA; UDMA; GPDM; HEMA; bis-GMA; filler; photo initiator	1.8[2]	4677483

Group IV Prime&Bond® NT 2-step Etch-and-rinse adhesive	Dentsply DeTrey, Konstanz, Germany	-Di-and trimethacrylate resins -PENTA -Photoinitiators -Stabilizers -Acetone -Nanofillers	2.2 [2]	1206000730
Group V Clearfil™ SE Bond 2-step Self-etch Adhesive	Kuraray, Okayama, Japan	Primer: 10-MDP; HEMA; hydrophilic aliphatic dimethacrylate; dl-camphorquinone; N,N-Diethanol-p-toluidine; water Adhesive: Bis-GMA; 10-MDP; HEMA; hydrophobic aliphatic dimethacrylate; dl-camphorquinone; N,N-Diethanol-p-toluidine; colloidal silica	1.9[2]	041931

BHT: Butylated hydroxyl toluene; **Bis-GMA:** Bisphenol A diglyciyl methacrylated; **GPDM:** glycerol phosphate dimethacrylate; **HEMA:** 2-hydroxyethl methacrylate; **PAAM:** Phthalic acid monoethyl methacrylated; **PEM-F:** Mono fluoro phosphazene modified methacrylate; **PENTA:** Dipentaerythritol pentaacrylate phosphate; **PYRO-EMA:** Phosporic acid modified methacrylate; **MDP:** methacryloyloxydecyl; **TEGDMA:** triethylene glycol dimethacrylate; **UDMA:** Urethane dimethacrylate;

The bonding and light-curing procedures were carried out as recommended by each manufacturer (Table II).

Table II: Adhesive application procedures (according manufacture recomentations).

Group/ Adhesive system	**Application procedure**
I - Xeno® V+	Apply actively adhesive for 20 sec; air-drying for 5 sec; light-curing for 10 sec.
II - Xeno® III	Mixing equal amount of Liquid A and B for 5 sec; apply actively for at least 20 sec; air-drying; light-curing for 10 sec
III - OptiBond™ FL	Apply 37.5% phosphoric acid (Kerr Gel Etchant®) for 15 sec; rinse for 15 sec; gently air-dry; apply primer actively for 15 sec; gently air-dry for 5 sec; apply the adhesive for 15 sec air-dry for 3 sec; light-curing for 20 sec.
IV - Prime & Bond® NT	Apply 36% phosphoric acid for 15 sec; Spray and rinse with water for 15 sec; blot dry conditioned areas; apply adhesive and leave the surface wet for 20 sec; gently air-dry for at least 5 sec; Polymerize for 10 sec; apply a second layer of adhesive in similar way.
V - Clearfil™ SE Bond	Apply primer for 20 sec; mild air stream; apply bond; gentle air stream; light-curing for 10 sec.

Following the bonding procedures, the crowns of the cut teeth were reconstructed with three incremental layers (1.5 mm) of light-cured microhybrid composite resin Esthet.X® HD A2 (DentsplyDeTrey, Konstanz, Germany) (Table III). Each layer was polymerized separately for 10 seconds followed by an extra-time final polymerization of 60 seconds (Bluephase®, Ivoclar Vivadent, Lichenstein).

Table III: Composite resin; manufacturer, composition, filler and batch no.

Composite	Manufacturer	Composition	Filler	Batch no.
Esthet•X® HD A2 Microhybrid	Dentsply DeTrey, Konstanz, Germany	Bis-GMA adduct Bis-EMA adduct TEGDMA	Ba-F-Al-B-Si-glass Nanofiller sílica (77wt%; 60 vol%)	1006292

Bis-GMA: Bisphenol A dimethacrylate; **Bis-EMA**: Bisphenol A polyethylene glycol diether dimethacrylate; **TEGDMA:** Triethyleneglycol dimethacrylate

Immediately after composite curing, the teeth were kept intact and stored in distilled water at 37ºC during 7 days (Heraeus BK 6160, Kelvitron® Kp, Wehrheim, Germany).

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.[20], to produce dentin-composite resin sticks with a sectional square area of approximately 1.37mm^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) (figure 1). Finally, the roots were cut from the crown approximately 2 mm bellow the cementoenamel junction releasing the dentin/composite sticks which were then checked under a optical microscope (M300, Leica, Switzerland) at 40-fold magnification in order to exclude samples with defects. The number of stick specimens obtained per each group was: Group I (Xeno® V+) n= 27; Group II (Xeno® III) n=24; Group III (OptiBond™ FL) n=41; Group IV (Prime & Bond® NT) n=40 and Group V (Clearfil™ SE Bond) n= 37.

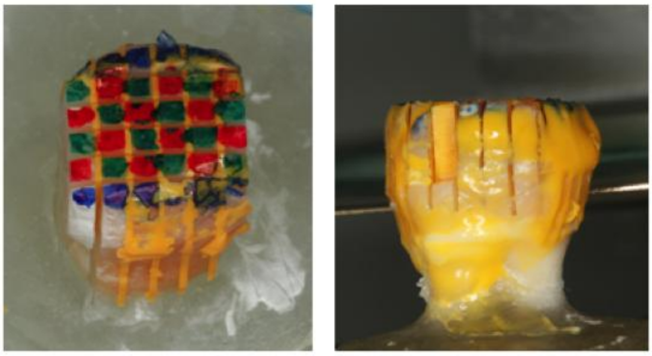

Figure 1: Representative images of oclusal and vestibular view of the teeth, before the final root section.

Microtensile bond strength testing

Each stick was bonded to a microtensile sample holder with cyanoacrylate adhesive (Permabond® 735, PermabondInternational Co, Englewood, NJ) 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-I, Shimadzu Corporation, Kyoto, Japan) at a 0.5 mm/min speed and the maximum load (in MPa) at failure was record.

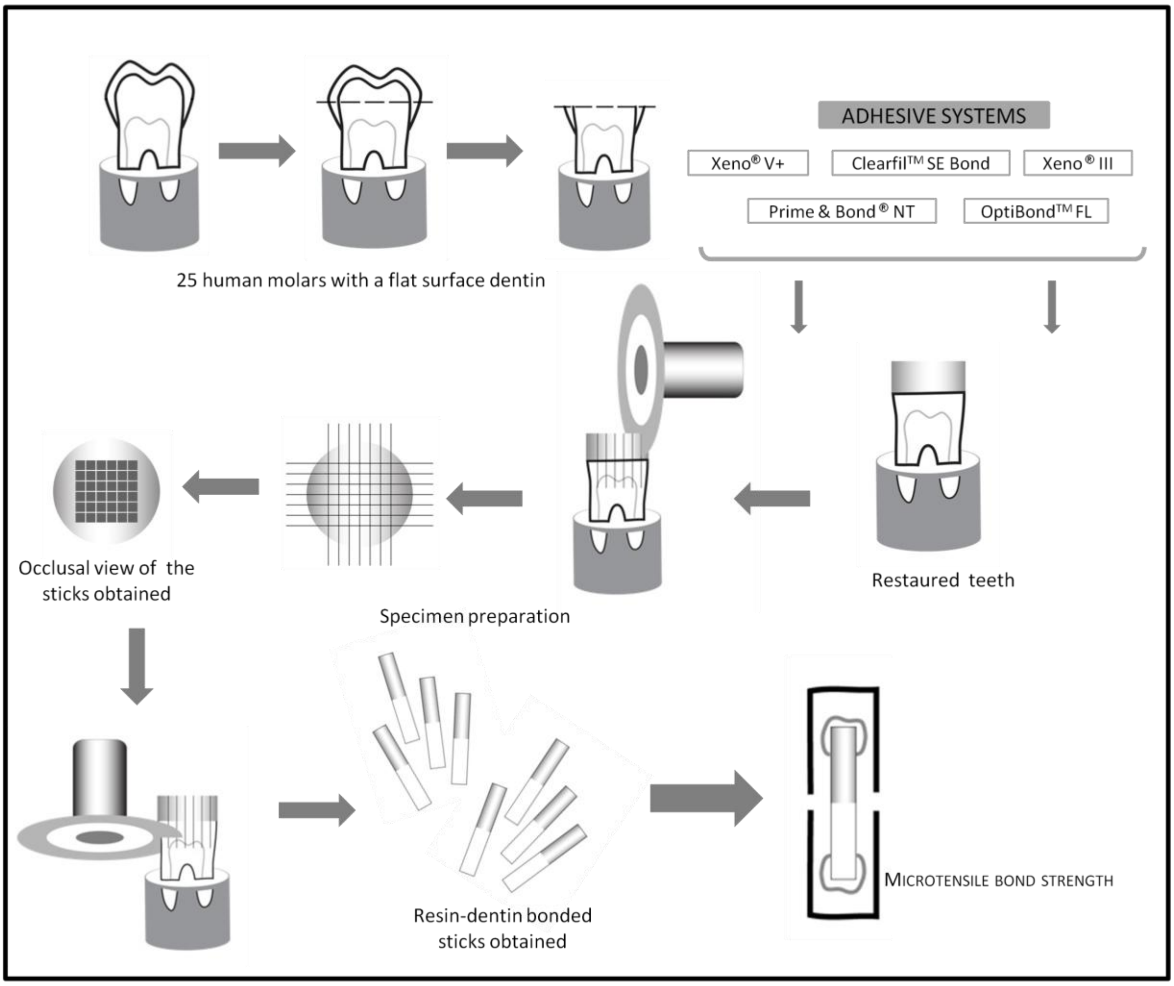

Figure 2: Esquematic diagram from study

After the microtensile testing, the fractured sticks were examined with a optical microscope (M300, Leica, Switzerland) at a 40-fold magnification and the mode of failure was recorded.

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

Ultramorphology analysis of dentin substrate by SEM

Two extra dentin disks of 1mm in thickness were obtained by means of two parallel sections of a molar crown (Accutom 5, Struers, Ballerup, Denmark) and then wet-grounded with a sequence of 240-, 400- and 600-grit silicon-carbide sandpaper in circular motion for 60 seconds to obtain a uniform smear layer, fixed in 2.5% glutaraldehyde within a PBS solution for 24 hours and, finally, divided in four samples according the different dentin conditioning preconized for each adhesive system: (1) 36% phosphoric acid; (2) Clearfil^{TM} SE Bond primer; (3) Xeno$^{®}$ III primer and (4) Xeno$^{®}$ V+. The samples were dehydrated in ascending ethanol series of 50%, 75%, 95% and 100% for at least 2 minutes per step, except the last one during for 4 minutes and immersed in hexamethyldisilazane (HMDS) until complete solvent evaporation.

After chemical dehydration, the specimens were mounted on a specimen aluminium stub using carbon adhesive, sputter-coated with gold-palladium (Polaron E-5000 Sputter-Coater, Polaron Equipment Lta, Watford, U.K.) before SEM analysis with a Hitachi S-4100 microscope (Hitachi, Tokyo, Japan).

Results

Figure 3 and table IV shows the results of the microtensile bond strength of the five adhesive systems tested.

OptiBond™ FL, Clearfil™ SE Bond and Prime & Bond® NT had the best performance in microtensile bond strength test. "Spontaneous" interfacial debonding occurred in 11 cases of the Xeno® V+ group, which presented inferior mean values. Additional similar pre-test failures were only found in two samples of Xeno® III.

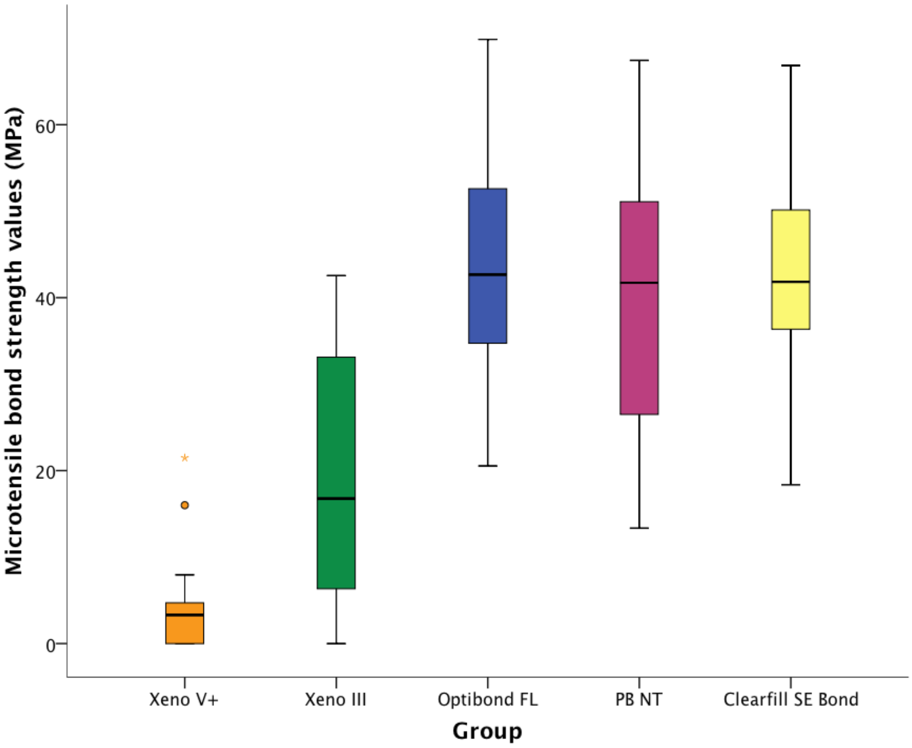

Figure 3: Box plot graphic for microtensile bond strength values distribution within groups

Table IV: Descriptive statistics for microtensile bond strength values of groups. Mean, standard deviation (SD), minimum and maximum values in MPa. Same upper case letters are not satistically different.

Group	Adhesive Systems	n	Mean ±SD	Min	Max	95% CI
I	Xeno® V+	27	3.70±5.01^{A}	0.00	21.48	[1.72, 5.68]
II	Xeno® III	24	18.94±13.87^{B}	0.00	42.55	[13.08,24.80]
III	OptiBond™ FL	39	43.29±12.74^{C}	20.54	69.87	[39.16,47.42]
IV	Prime & Bond® NT	40	39.64±15.06^{C}	13.35	67.44	[34.83,44.46]
V	Clearfil™ SE Bond	37	42.80±10.65^{C}	18.36	66.84	[39.24,46.35]

Mean adhesion values for the five groups were compared using one-way ANOVA setting the significance level at $\alpha=0.05$ and considering zero as the value for pre-test failures. Post-hoc analysis was performed with Tukey HSD multiple comparisons test. There were no real outliers and the data was normally distributed for all groups ($p>0.05$) except Xeno® V+ ($p=0.001$), as assessed by box plot and Kolmogorov-Smirnov test. There was no 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 groups of adhesives $F(4,162)=62.50$, $p<0.01$, $\omega^2=158.99$. Bond strength values increased from the Xeno® V+ group (3.70±5.01 MPa), to Xeno® III (18.94±13.87 MPa), to Prime & Bond® NT (39.64±15.06 MPa), to Clearfil™ SE Bond (42.80±10.65 MPa) and to OptiBond™ FL (43.29±12.74 MPa), in that order.

Post-hoc analysis revealed that the increase from Xeno® V+ to Xeno® III (15.24, 95% CI [5.78,24.69]), from Xeno® V+ to Prime & Bond® NT (35.94, 95% CI [27.55,44.34]), from Xeno® V+ to Clearfil™ SE Bond (39.10, 95% CI [30.57,47.63]) and from Xeno® V+ to OptiBond™ FL (39.59, 95% CI [31.16,48.03]) was statistically significant ($p<0,01$). As shown in Table V increases in mean bond strength values from Xeno® III to Prime & Bond® NT, Clearfil™ SE Bond and OptiBond™ FL groups were also statistically significant.

There was a statistically significant difference between means ($p<0.05$), thus we can reject the null hypothesis.

Table V: Multiple comparisons for groups I and II (Tukey HSD test). Mean difference of microtensile bond strength values between groups in MPa

(I) Group	(J)	Mean Difference (I-J)	Std. Error	p	95% Confidence Interval	
					Lower Bound	Upper Bound
Xeno® V+	Xeno® III	-15.24*	3,43	,000	-24,69	-5,78
	Optibond™ FL	-39.59*	3,06	,000	-48,03	-31,16
	PB® NT	-35.94*	3,04	,000	-44,34	-27,55
	Clearfill™ SE Bond	-39.10*	3,09	,000	-47,63	-30,57
Xeno® III	Xeno® V+	15.24*	3,43	,000	5,78	24,69
	Optibond™ FL	-24.36*	3,17	,000	-33,10	-15,61
	PB® NT	-20.70*	3,15	,000	-29,41	-12,00
	Clearfill™ SE Bond	-23.86*	3,20	,000	-32,69	-15,02

The crosstabulation, observed and expected frequencies for each cell of the design are found in the Failure*Group Crosstabulation table (Table VI), as shown below:

Table VI Failures mode percentage of the debonded specimens for each group: **Ad -** Adhesive failure **DC -** Dentin cohesive failure; **CC** - Composite cohesive failure; **Mix -** Mixed failure;

Failure * Group Crosstabulation

			Group					
			Xeno® V+	Xeno® III	Optibond™ FL	PB® NT	Clearfill™ SE Bond	Total
Failure	Ad	Count	16^{a}	13^{b}	0_{c}	4_{c}	2_{c}	35
		% within Failure	45,7%	37,1%	0,0%	11,4%	5,7%	100,0%
	DC	Count	0_{a}	3_{a}	2_{a}	0_{a}	4_{a}	9
		% within Failure	0,0%	33,3%	22,2%	0,0%	44,4%	100,0%
	CC	Count	0_{a}	1_{a}	31_{b}	22_{b}	25_{b}	79
		% within Failure	0,0%	1,3%	39,2%	27,8%	31,6%	100,0%
	Mix	Count	0_{a}	5_{a}	6_{a}	14_{a}	6_{a}	31
		% within Failure	0,0%	16,1%	19,4%	45,2%	19,4%	100,0%
Total		Count	16	22	39	40	37	154
		% within Failure	10,4%	14,3%	25,3%	26,0%	24,0%	100,0%

Each subscript letter denotes a subset of Group categories whose column proportions do not differ significantly from each other at the .05 level.

Chi-square test for association was conducted between failure type and adhesive system. There was a statistically significant association between failure type and adhesive system, X^2 (12)= 112.64, $p<0.05$

There was a moderately strong association between failure type and adhesive system, V= 0.49, $p<0.01$. Adhesive failures were associated with both Xeno® V+ and Xeno® III. Cohesive composite fractures were associated with both OptiBond™ FL, Prime & Bond® NT and Clearfil™ SE Bond.

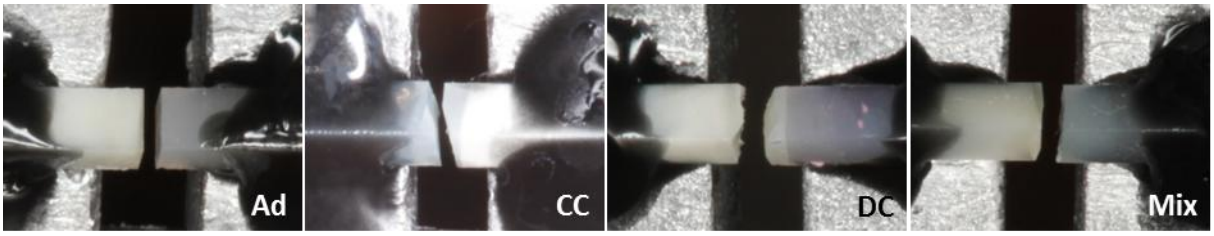

Figure 4: Representative images of the different failure modes: **Ad -** Adhesive failure; **CC -** Composite cohesive failure; **DC -** Dentin cohesive failure; **Mix -** Mixed failure.

SEM observations

Figure 5 is a SEM representative image of the smear layer adhered to dentin surface and partially occluding the dentinal tubules before different adhesive dentin conditionings.

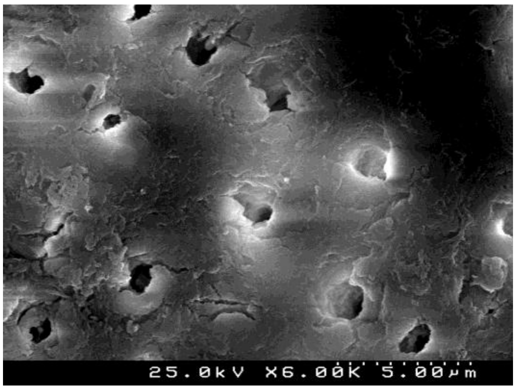

Figure 5: SEM representative image(6000x) illustrating the smear layer covered dentin.

Concerning the ultra morphology analysis of the adhesive conditioned dentin, the SEM analysis showed different patterns (Figure 6).

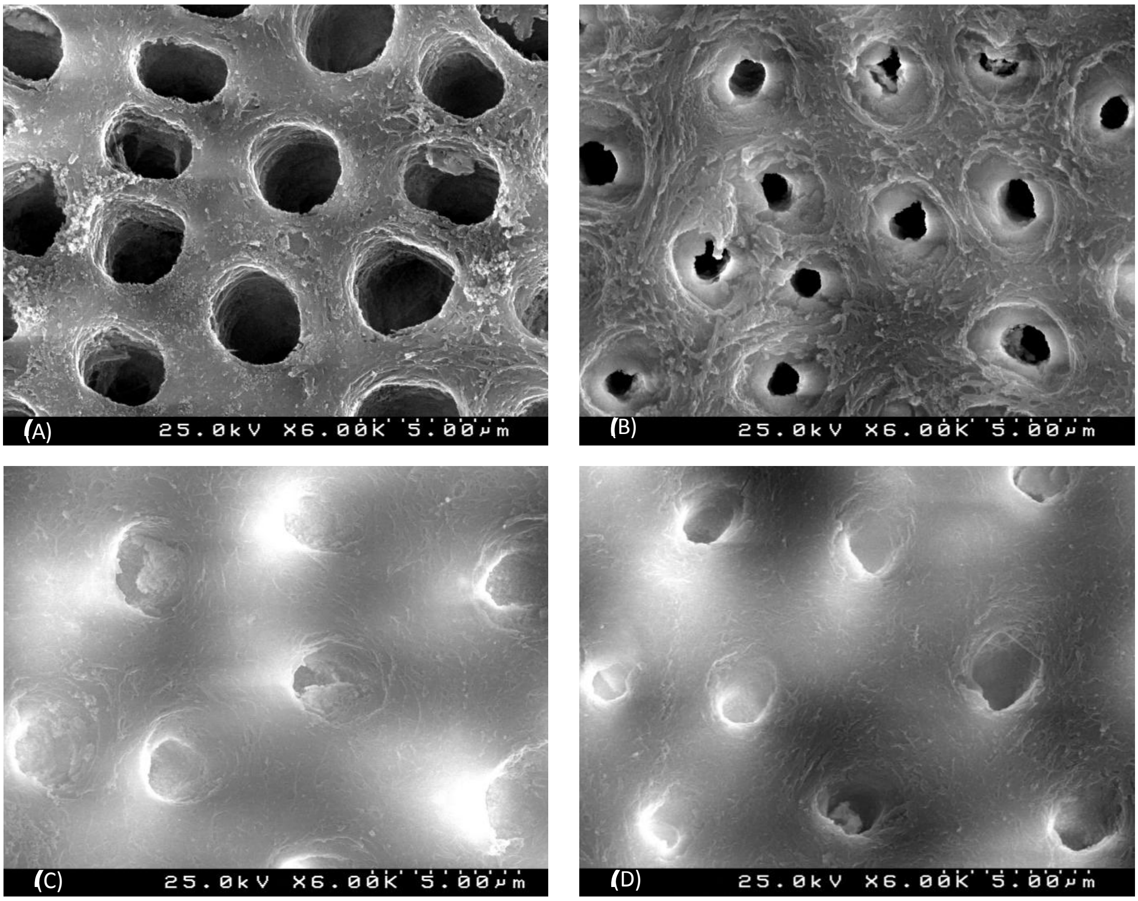

Figure 6: Representative SEM images (6000X) of dentin treated with: (A)- 36% phosphoric acid for 15 seconds and rinse with water; (B)- Clearfil™ SE Bond primer; (C)- Xeno® III; (D)- Xeno® V+.

The analysis of the dentin disks under SEM showed that after etching with 36% phosphoric acid smear layer was completely removed and subjacent dentin and dentinal tubule orifices were visible and their input was expanded.

The primer of two-step self-etching adhesive system, Clearfil™ SE Bond, removed most smear plugs and showing the underlying dentinal tubules and also a partially visible, dissolved smear layer in the intertubular area.

Unlike to phosphoric acid and Clearfil™ SE Bond primer, the patterns of dentin surface created by one-step self-etch adhesive systems Xeno® III and Xeno® V+ showed that smear plugs were not removed, or only partially, and a partially dissolved smear layer was visible in the intertubular area.

Discussion

Today the current challenge in adhesive dentistry is to ensure the long-term success, making the adhesive-tooth interfaces more resistant against aging[2]. One of the most important issues of recent adhesive materials is its durability[21], which seems to be dependent either on the adhesive's formulation and the bonding strategy[21].

The transition between the restorative material and the dental hard tissue must be continuous to increase the survival probability of the restoration[22]. In spite of adhesive bond strengths to enamel are predictable, satisfactory and stable when etch-and-rinses systems are employed[23, 24] bonding to dentin is a much more complex issue, due to the nature of this substrate[24], namely its higher organic content and the presence of fluid and odontoblastic processes in the tubules[23]. The long-term durability appears to be influenced by substrate and polymer stability, activity of metal matrix proteins (MMPs) and the ongoing decalcification by bacterial generated acids[25].

No internationally standardization test protocols yet exists for the testing adhesives systems[26]. Therefore, variable methods and parameters have been employed by the different laboratories making difficult the comparison across studies[27].

The interface between restoration and tooth is exposed to different challenges in oral cavity such as forces that act in different directions and simultaneously[2] or thermal oscillations that can induce cumulative stresses and progressive interface degradation[9]. By definition, the ideal bond strength test should be easy to perform, low technique-sensitivity, relatively fast, unsophisticated and inexpensive[7], and, most important, capable to simulate biomechanics conditions of restored teeth. Bond strength testing is the method most used for the assessment of bonding effectiveness to enamel and dentin[28, 29] including different mechanical methods[30]. These tests are used to evaluate the ability of a restorative material or dentin bonding system to establish a bond to a biological substrate[22].

Generally, bond strength can be measured by macro and micro test set-ups, basically depending upon the bonded size area[7]. The bond strength tests results can be affected by several parameters, like, operator, research group/institute, adhesive system, adhesive class, substrate preparation, substrate origin and composites flexural modulus[31]. The characteristic of the bonding substrate plays a major role on the quality of adhesion[6]. Clinically relevant substrates include caries-affected, caries infected, sclerotic, deep and bur cut dentin[32]. In the present study bond strengths were measured under a sound dentin substrate that always play a important role in surrounding walls of cavities design. Besides the same composite resin was used combined with each adhesive system studied for cutting off the possible influence of resin composite modulus development.

The shear bond-strength test is the most commonly used[30], namely between manufacturers, because it is easy and fast to perform[7]. However, some problems have been related to the shear bond strength test, as unrealistic stresses are produced within the reaction zone[31]. Microtensile bond strength test allows measurements of the tensile bond strength on very small surfaces, about 1 mm[2, 20, 33]. This method allows multiple specimens to be prepared from each tooth[34], measuring bond strength at critical areas[31], with a more uniform stress distribution in the reaction zone and a higher reliable correlation with clinical retention loss[31].

There are several critical factors that influence the results of the microtensile bond strength test, such as diameter of the stick, type of jig, trimming[35] and storage of bonded specimens in water[31].

In this test, the loading force passes through the tooth substrate and composite resin before the adhesive interface[36]. Thus, the subsequent stress concentration could explain the frequent cohesive failure in the tooth substrate[36], which not reflect the true bond strength[26].

Microtensile bond strength tests have a number of advantages, such as: more adhesive failures and fewer cohesive failures[27, 29]; measurement of higher interfacial bond strengths[27,29]; allows testing on very small surfaces[27,29, 37] or in irregular surfaces[27,29]; means and variances can be calculated for single teeth[27, 29]; and facilities examination of failed bonds by scanning electron microscopy[29,27]. However, some disadvantages were also described for the microtensile bond strength test, as the labour intensity, technical demand and dehydration potential of these smaller samples[27].

In the present study, the best performing adhesive systems was the 3-step etch-and-rinse OptiBond™ FL (43.29±12.74), followed by 2-step self-etch Clearfil SE™ Bond (42.80±10.65) and Prime & Bond® NT (39.64±15.06) without no statistical differences found between them. OptiBond™ FL has already showed very favourable laboratory[38, 39] and clinical performance[28, 40, 41] Pashley *et al*[4] referred that 3-steps etch-and-rinse adhesives are more durable than 2-step etch-and-rinse adhesives, due to the first one has the advantage and opportunity to use each step for essential multipurpose objectives.

OptiBond™ FL bonding resin composition has cross-linking monomers. The relative amounts of Bis-GMA, TEGDMA and UDMA presents is this adhesive system may have a meaningful influence on the viscosity of the uncured adhesive resin and on the mechanical properties of the cured resin[42]. TEGDMA has high flexibility which is compensated by the rigidity of Bis-GMA[42]. Some studies that used OptiBond™ FL presented very scatter bond strength values. Phrukkanon *et al*[43] (1998) obtained 20.2±5.0 MPa which is lower comparing with the present study. Conversely, Heintze & Zimmerli[26] (2011) in a study about the relevance of in vitro tests of different adhesive systems, registered 48.0±13.7 MPa to OptiBond™ FL, which is similar to those obtained in the present work. Along with two-step self-etch adhesives, three-step etch and rinse adhesives are considered the *gold standards* for dentin adhesion.

Presenting also good performance, Prime & Bond® NT, contains an acidic phosphonated monomer (PENTA) which can interact with calcium ions left on dentin surface[21]. This adhesive system is filled with nanoparticles that may help to establish a thicker and more uniform resin film thickness that stabilizes the hybrid layer[44]. Prime&Bond® NT had a mean microtensile bond strength of 39.64MPa. The performance of this adhesive has been evaluated by other authors which registered lowers values than de present study[37,45].

Clearfil SE™ Bond, though belonging to the group of "mild" self-etch adhesives[9], also had a high performance concerning bond strengths. This adhesive system was the only self-etch obtaining microtensile bond strength values comparable to those etch-and-rinse adhesives and no statistical differences were found between them.

Osorio *et al.*[21] (2008) stated that high percentage of camphorquinone in this adhesive might improve the degree of polymerization and the presence of 10-methacryloxydecyl dihydrogen phosphate (10-MDP) which can contribute to the higher bond stability due to its chemical adhesion with tooth tissues[42]. In fact, this 10-MDP molecule can chemically bond to calcium of hidroxiapatite, according to the adhesion decalcification concept, forming a stable calcium-phosphate salt, along with only a limited surface-decalcification effect. 'Mild' self-etch adhesives indeed only superficially interact with dentin, and hardly dissolve hidroxiapatite crystals, but rather keep them in place, forming a thin submicron hybrid layer[3]. In this study, microtensile bond strength results for Clearfil™ SE Bond were 42.80±10.65 MPa. In the literature, similar results were found to this adhesive system by other authors[15,26].

Clinicians prefer materials with an easy, simplified application. However, these simplified adhesives can be associated to a less optimal clinical effectiveness[9].

In this present study the adhesive system with lowest performance was the Xeno® V+ (DentisplyDeTrey, Konstanz, Germany), followed by the Xeno® III (DentisplyDeTrey, Konstanz, Germany). Xeno® V+ presented a mean of 3.70±5.01 (MPa) and 11 pre-test failures within 27 specimens in total. Another study, by Nikhil *et al.*[46] (2011), also demonstrated a lower performance for Xeno® V+ and referred that the adhesive system bond strength can be associated to absence and presence of HEMA, which is absent in case of Xeno® V+ (HEMA-free). In adequate concentration, this monomer can contribute for bond strength due to its hydrophilic nature that makes it an excellent adhesion-promoting monomer and by enhancing wetting of dentin. Nevertheless, HEMA-free one-step adhesives are complex blends of hydrophilic/hydrophobic ingredients, water and solvents, prone to phase separation, which can account partially for their lower bonding effectiveness[47]. On the other hand, Xeno® V+ adhesive system contains tertiary butanol, which is related to a more stable formulation than those containing ethanol, according El-kholany *et al*[48]. Although a long shelf-life has been attributed for this adhesive by the manufacturer, possible alterations in Xeno® V+ components can also explain the lowest results reported in this study.

In this study Xeno® III also presented, comparatively, lower bond strength values. In high amounts, HEMA, which is a Xeno® III component, may have deteriorating effects on the mechanical properties of the resulting polymer[42]. For this adhesive system, Loguercio *et al*.[49] reported a higher bond strength result, 28.3MPa, while Bortolloto *et al*.[50] registered a mean of 23.8 MPa, and Amaral *et al*.[51] showed a mean of 19.9MPa in microtensile bond strength for this one-step self-etch adhesive.

Simplified one-step self-etch adhesive system does not provide the formation of a high quality hybrid layer compared to 2-step self-etching primers and conventional etch & rinse adhesives[15]. One-step self-etch adhesives composition are a complex mixture of hydrophobic acid monomer, hydrophilic resins, solvent and water[15,29,52]. The lack of hydrophobic resins for hybrid layer formation[15] and, consequently due to their hydrophilic nature, these adhesives systems may act as permeable membranes and absorb significant amounts of water, even when polymerized[52] which can compromise bond strength to dentin and influence the failure mode[15]. Moreover, monomer degradation has several adverse effects on the performance of dental adhesives, predominantly by the deterioration of bond strength and inducing morphological changes at the adhesive/dentin interface[53].

In the presented study, the mode of failure was related with the adhesive system. The adhesive failure was associated to low bond strengths values, meaning for the Xeno® V+ and Xeno® III. On the other hand, the higher bond strength values were correlated with cohesive failures that occurred in the best performance adhesive systems OptiBond™ FL, Clearfil™ SE Bond and Prime &Bond® NT.

Similar results were reported in other study. Ceballos *et al.*[44] (2003) correlated the bond strength values with mode of failure. Low bond strengths were associated with adhesive failures, while cohesive fractures were seen at higher bond strengths.

Scherrer *et al.*[54] (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, but correlated significantly well with greater adhesion values. 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 resin or dentin.

Statistical analysis showed 11 pre-test failures within Group I (Xeno® V+) and 2 for Group II (Xeno® III). The high number of the pre-test failures represented an additional point for the scatter in microtensile bond strength results[54]. These values can be treated by different manner by researchers[31,54]. In the present study they were considered as zero in the statistical analysis, which explain the decrease in mean values and the increase in standard deviation, but reflects truly the concern about the weakness of the produced adhesive interface[55].

Concerning the 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.

Conclusions

Within the limitations of this in vitro study, it can be concluded that:

- Etch-and-rinse adhesives and two-step self-etch adhesives showed higher microtensile bond strength values than one-step self-etch adhesives.

- Adhesive failure mode was correlated to lower bond strength results, while the cohesive failures are more common with higher adhesion values.

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References

1. Cardoso MV, de Almeida Neves A, Mine A, Coutinho E, Van Landuyt K, De Munck J, et al. Current aspects on bonding effectiveness and stability in adhesive dentistry. Aust Dent J 2011;56 Suppl 1:31-44.
2. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, et al. Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges. Oper Dent 2003;28(3):215-35.
3. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt KL. State of the art of self-etch adhesives. Dent Mater 2011;27(1):17-28.
4. Pashley DH, Tay FR, Breschi L, Tjaderhane L, Carvalho RM, Carrilho M, et al. State of the art etch-and-rinse adhesives. Dent Mater 2011;27(1):1-16.
5. Perdigao J. Dentin bonding-variables related to the clinical situation and the substrate treatment. Dent Mater 2010;26(2):e24-37.
6. Spencer P, Ye Q, Park J, Topp EM, Misra A, Marangos O, et al. Adhesive/Dentin interface: the weak link in the composite restoration. Ann Biomed Eng 2010;38(6):1989-2003.
7. Van Meerbeek B, Peumans M, Poitevin A, Mine A, Van Ende A, Neves A, et al. Relationship between bond-strength tests and clinical outcomes. Dent Mater 2010;26(2):e100-21.
8. Pashley DH, Carvalho RM. Dentine permeability and dentine adhesion. J Dent 1997;25(5):355-72.
9. Peumans M, Kanumilli P, De Munck J, Van Landuyt K, Lambrechts P, Van Meerbeek B. Clinical effectiveness of contemporary adhesives: a systematic review of current clinical trials. Dent Mater 2005;21(9):864-81.
10. Van Meerbeek B, De Munck J, Mattar D, Van Landuyt K, Lambrechts P. Microtensile bond strengths of an etch&rinse and self-etch adhesive to enamel and dentin as a function of surface treatment. Oper Dent 2003;28(5):647-60.
11. Van Meerbeek B, Yoshida Y, Van Landuyt K. Fundamentals of Operative Dentistry. A contemporary approach. 3rd edn. Chicago: Quintessence Publishing 2006:183-260.
12. Van Meerbeek B, Inokoshi S, Braem M, Lambrechts P, Vanherle G. Morphological aspects of the resin-dentin interdiffusion zone with different dentin adhesive systems. J Dent Res 1992;71(8):1530-40.
13. Langer A, Ilie N. Dentin infiltration ability of different classes of adhesive systems. Clin Oral Investig 2013;17(1):205-16.

14. Yoshida Y, Nagakane K, Fukuda R, Nakayama Y, Okazaki M, Shintani H, et al. Comparative study on adhesive performance of functional monomers. J Dent Res 2004;83(6):454-8.
15. De Goes MF, Giannini M, Di Hipolito V, Carrilho MR, Daronch M, Rueggeberg FA. Microtensile bond strength of adhesive systems to dentin with or without application of an intermediate flowable resin layer. Braz Dent J 2008;19(1):51-6.
16. M.Junior M, P.Rocha E, B.Anchieta R, Archangelo CM, AntonioLuersen M, . Etch and rinse versus self-etching adhesives systems: Tridimensional micromechanical analysis of dentin/adhesive interface. InternationalJournalofAdhesion&Adhesives (2012);114–119
17. Sundfeld RH, Valentino TA, de Alexandre RS, Briso AL, Sundefeld ML. Hybrid layer thickness and resin tag length of a self-etching adhesive bonded to sound dentin. J Dent 2005;33(8):675-81.
18. Tay FR, Pashley DH. Dental adhesives of the future. J Adhes Dent 2002;4(2):91-103.
19. Dentsply D. Xeno® V+ Scientific Compendium.
20. 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. Dent Mater 1994;10(4):236-40.
21. Osorio R, Pisani-Proenca J, Erhardt MC, Osorio E, Aguilera FS, Tay FR, et al. Resistance of ten contemporary adhesives to resin-dentine bond degradation. J Dent 2008;36(2):163-9.
22. Heintze SD. Systematic reviews: I. The correlation between laboratory tests on marginal quality and bond strength. II. The correlation between marginal quality and clinical outcome. J Adhes Dent 2007;9 Suppl 1:77-106.
23. Dias WR, Pereira PN, Swift EJ, Jr. Effect of bur type on microtensile bond strengths of self-etching systems to human dentin. J Adhes Dent 2004;6(3):195-203.
24. Leloup G, D'Hoore W, Bouter D, Degrange M, Vreven J. Meta-analytical review of factors involved in dentin adherence. J Dent Res 2001;80(7):1605-14.
25. Salz U, Bock T. Testing adhesion of direct restoratives to dental hard tissue - a review. J Adhes Dent 2010;12(5):343-71.
26. Heintze SD, Zimmerli B. Relevance of in vitro tests of adhesive and composite dental materials. A review in 3 parts. Part 3: in vitro tests of adhesive systems. Schweiz Monatsschr Zahnmed 2011;121(11):1024-40.
27. Armstrong S, Geraldeli S, Maia R, Raposo LH, Soares CJ, Yamagawa J. Adhesion to tooth structure: a critical review of "micro" bond strength test methods. Dent Mater 2010;26(2):e50-62.

28. De Munck J, Mine A, Poitevin A, Van Ende A, Cardoso MV, Van Landuyt KL, et al. Meta-analytical review of parameters involved in dentin bonding. J Dent Res 2012;91(4):351-7.

29. Hamouda IM, Samra NR, Badawi MF. Microtensile bond strength of etch and rinse versus self-etch adhesive systems. J Mech Behav Biomed Mater 2011;4(3):461-6.

30. Burke FJ, Hussain A, Nolan L, Fleming GJ. Methods used in dentine bonding tests: an analysis of 102 investigations on bond strength. Eur J Prosthodont Restor Dent 2008;16(4):158-65.

31. Heintze SD. Clinical relevance of tests on bond strength, microleakage and marginal adaptation. Dent Mater 2013;29(1):59-84.

32. Carvalho RM, Manso AP, Geraldeli S, Tay FR, Pashley DH. Durability of bonds and clinical success of adhesive restorations. Dent Mater 2012;28(1):72-86.

33. Pashley DH, Sano H, Ciucchi B, Yoshiyama M, Carvalho RM. Adhesion testing of dentin bonding agents: a review. Dent Mater 1995;11(2):117-25.

34. Pashley DH, Carvalho RM, Sano H, Nakajima M, Yoshiyama M, Shono Y, et al. The microtensile bond test: a review. J Adhes Dent 1999;1(4):299-309.

35. Poitevin A, De Munck J, Van Landuyt K, Coutinho E, Peumans M, Lambrechts P, et al. Critical analysis of the influence of different parameters on the microtensile bond strength of adhesives to dentin. J Adhes Dent 2008;10(1):7-16.

36. 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. Dent Mater 2010;26(9):848-54.

37. de Castro FL, de Andrade MF, Duarte Junior SL, Vaz LG, Ahid FJ. Effect of 2% chlorhexidine on microtensile bond strength of composite to dentin. J Adhes Dent 2003;5(2):129-38.

38. Peutzfeldt A, Asmussen E. Influence of eugenol-containing temporary cement on bonding of self-etching adhesives to dentin. J Adhes Dent 2006;8(1):31-4.

39. 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. Oper Dent 2010;35(1):94-104.

40. Bogosian A, Drummond J, Lautenschlager E. Clinical evaluation of a dentin adhesive system: 13 year results. J Adhes Dent 2007;86.

41. Peumans M, De Munck J, Van Landuyt KL, Poitevin A, Lambrechts P, Van Meerbeek B. A 13-year clinical evaluation of two three-step etch-and-rinse adhesives in non-carious class-V lesions. Clin Oral Investig 2012;16(1):129-37.

42. 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.

43. Phrukkanon S, Burrow MF, Tyas MJ. Effect of cross-sectional surface area on bond strengths between resin and dentin. Dent Mater 1998;14(2):120-8.

44. 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. J Dent 2003;31(7):469-77.

45. Mazur R, Almeida J, Martin J, Soares P, Caldas D, Souza E. Microtensile Bond Strength of Adhesive systems of Single and Multiple steps. Rev. Clín. Pesq. Odontologia, Curitiba 2009;5:89-94.

46. Nikhil V, Singh V, Chaudhry S. Comparative evaluation of bond strength of three contemporary self-etch adhesives: An ex vivo study. Contemp Clin Dent 2011;2(2):94-7.

47. 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. J Dent Res 2005;84(2):183-8.

48. El-kholany NR, Abielhassan MH, Elembaby AE, Maria OM. Apoptotic effect of different self-etch dental adhesives on odontoblasts in cell cultures. Arch Oral Biol 2012;57(6):775-83.

49. Loguercio AD, Stanislawczuk R, Mena-Serrano A, Reis A. Effect of 3-year water storage on the performance of one-step self-etch adhesives applied actively on dentine. J Dent 2011;39(8):578-87.

50. Bortolotto T, Mileo A, Krejci I. Strength of the bond as a predictor of marginal performance: an in vitro evaluation of contemporary adhesives. Dent Mater 2010;26(3):242-8.

51. do Amaral RC, Stanislawczuk R, Zander-Grande C, Michel MD, Reis A, Loguercio AD. Active application improves the bonding performance of self-etch adhesives to dentin. J Dent 2009;37(1):82-90.

52. Knobloch LA, Gailey D, Azer S, Johnston WM, Clelland N, Kerby RE. Bond strengths of one- and two-step self-etch adhesive systems. J Prosthet Dent 2007;97(4):216-22.

53. Salz U, Bock T. Adhesion performance of new hydrolytically stable one-component self-etching enamel/dentin adhesives. J Adhes Dent 2010;12(1):7-10.

54. Scherrer SS, Cesar PF, Swain MV. Direct comparison of the bond strength results of the different test methods: a critical literature review. Dent Mater 2010;26(2):e78-93.

55. Marquezan M, da Silveira BL, Burnett LH, Jr., Rodrigues CR, Kramer PF. Microtensile bond strength of contemporary adhesives to primary enamel and dentin. J Clin Pediatr Dent 2008;32(2):127-32.