Universidade de Lisboa

Faculdade de Medicina Dentária

Effect of pre-etching on *in vitro* enamel bond strength of two adhesive systems: Universal Vs. Self-etch

Daniela Sofia de Gonçalves Rocha

Mestrado Integrado em Medicina Dentária

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Efeito do pré-condicionamento ácido nas forças de adesão em esmalte *in vitro* de dois sistemas adesivos: Universal Vs. Self-etch

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"Choose a job you love,
and you will never have to work a day in your life"
Confucius

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Resumo

Objetivos: O presente trabalho tem como objetivo avaliar o efeito do pré-condicionamento ácido nas forças de adesão em esmalte *in vitro* de dois sistemas adesivos: um adesivo universal e um self-etch de dois passos.

Materiais e Métodos: Neste estudo utilizaram-se 8 dentes molares humanos livres de cárie. Foram realizados cortes de maneira a hemissecionar as coroas dos dentes mesiodistalmente e estas foram aleatoriamente divididas em 2 grupos (n=8). Às superfícies em esmalte foram aplicados 2 sistemas adesivos distintos: Scotchbond Universal (3M ESPE Seefeld, Germany) segundo instruções do fabricante pela técnica total-etch; Clearfil SE Bond (Kuraray, Okayama, Japan) pela ténica total-etch, tendo sido restauradas com o compósito ENAMEL plus HRi (Micerium S.p.A. Avegno (GE) Italy) fotopolimerizados em três incrementos, cada um com 2mm. Os espécimenes foram armazenados em água destilada a 37ºC durante 24h e depois seccionados em palitos de aproximadamente 1mm^2 com um disco de diamante sob refrigeração com água, nas direções X e Y de maneira a obter palitos. Todas as amostras foram testadas até à fratura em testes de microtração, a uma velocidade de 1mm/minuto. A análise estatística foi feita utilizando o T-student. As fraturas foram observadas num estereomicroscópio e registadas.

Resultados: Os dois grupos estudados não apresentam diferenças estatisticamente significativas (p<0,05).

Conclusão: Um adesivo universal parece proporcionar forças de adesão em esmalte similares a adesivos self-etch de dois passos quando efectuado pré-condicionamento ácido.

Palavras-chave: Adesivos, Self-etch, Total-etch, Microtração, Enamel.

Abstract

Objectives: The purpose of this study was to evaluate the effect of pre-etching on *in vitro* enamel bond strength of two adhesive systems: one universal adhesive and one two-step self-etch.

Methods: In this study were used 8 caries-free human molars. The specimens were partially split in two halves and were assigned to two groups (n=8). On the enamel surfaces were applied two different adhesive systems: Scotchbond Universal (3M ESPE Seefeld, Germany) following manufacturer's instructions as a total-etch; Clearfil SE Bond (Kuraray, Okayama, Japan) applied as a total-etch. Build-ups were constructed with ENAMEL plus HRi (Micerium S.p.A. Avegno (GE) Italy) and cured in three increments of 2mm each. Specimens were kept in 37ºC destilated water for 24 hours and then sectioned with a slow-speed Diamond saw under water in X and Y directions to obtain bonded beams that were tested to failure in tension at a crosshead speed of 1 mm/minute. Statistical analyses were computed using T-student. The failures interfaces were observed under an optical microscope and registered.

Results: There were not statistically significant differences among the two groups.

Conclusions: A new universal adhesive system has similar bond strength than a two-step self-etch adhesive when pre-etching is performed.

Key-words: Adhesives, Self-etch, Total-etch, Microtensile, Enamel.

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Abreviations

E&R or TE – Etch-and-rinse or total-etch

SE or E&D – Self-etch adhesives

SBU – Scotchbond Universal Adhesive

CSE – Clearfil SE Bond Adhesive

pH – potential of hydrogen

µTBS – Microtensile bond strength

MDP - 10- Methacryloyloxydecyl dihydrogen phosphate

HEMA – 2-Hydroxyethyl methacrylate

Bis-GMA - Bisphenol A diglycidylmethacrylate

SD - Standard deviation

PA – Phosphoric acid

HA – Hidroxyapatite

1. Introduction

Bonding procedures to enamel play an important role in restorative and preventive dental procedures, and for many years, the bond obtained using phosphoric acid (PA) etching of enamel was the mainstay of such procedures (Erickson *et al.*, 2009).

The task of achieving an intimate adaptation between the restorative material and the dental substrate is different for enamel and dentin, since the bonding processes are not alike. That is, dentin is more humid and more organic than enamel, while enamel is composed of 96% hydroxyapatite (mineral) by weight, dentin contains more water and organic material, mainly type-I collagen (Asmussen and Uno, 1992). This makes bonding to dentin a challenge.

When tooth structure is cut with a bur or other instrument, the residual components form a "smear layer" of debris on the surface, this debris forms a uniform coating on enamel and dentin, reducing their permeability (Bowen *et al.*, 1984), making bonding even more challenging

Almost sixty years ago Buonocore (1955) reported the use of 85% phosphoric acid to improve the retention of an acrylic resin to enamel. The micromechanical nature of the interaction of dental adhesives with enamel is a result of the infiltration of resin monomers into the microporosities left by the acid dissolution of enamel and subsequent enveloping of the exposed hydroxyapatite crystals with the polymerized monomers within the pores on enamel surfaces (Swift *et al.*, 1995).

<u>Adhesives classification</u>

In spite of different classifications of adhesive systems, the current adhesion strategies depend exclusively on how dental adhesives interact with smear layer. One strategy involves total-etch or etch-and-rinse adhesives (TE or E&R), which remove the smear layer and the superficial hydroxyapatite through etching with a phosphoric acid gel that is later rinsed away. The second strategy involves self-etching or etch-and-dry adhesives (SE or E&D), containing acidic monomers, which will not be rinsed and will make the smear layer permeable without completely removing it (Perdigao, 2007; Tay and Pashley, 2002; Van Meerbeek *et al.*, 2003).

The other fundamental steps for adhesion, despite the differences in etching, are

priming and bonding that can be either separate or combined, depending on the adhesive system. So, the current classification of adhesives relies also on the number of the application steps (Van Meerbeek *et al.*, 2003). E&R adhesive systems can be either three- or two-step depending on whether the primer and the adhesive are separated or combined on a single bottle. Similarly self-etch adhesives can be either two- or one-step systems depending on whether the etching/primer agent is separated from the adhesive or combined with it to allow a single application procedure.

Etch-and-rinse/ Total-etch approach

This E&R technique is still the most effective approach of achieving efficient and stable bonding to enamel and basically only requires two steps. Selective dissolution of hydroxyapatite crystals through etching (commonly with a 30-40% phosphoric-acid gel) is followed by *in situ* polymerization of resin that is readily absorbed by capillary attraction within the created etch pits, thereby, enveloping individually exposed hydroxyapatite crystals (Van Meerbeek *et al.*, 2003). Most critical on the E&R approach is the priming step. When an acetone-based adhesive is used, the highly technique-sensitive *"wet-bonding"* technique is mandatory (Pashley *et al.*, 2011; Tay *et al.*, 1996). Otherwise, gentle post-conditioning air-drying of acid-etch dentin and enamel following a *"dry-bonding"* technique still guarantees effective bonding when a water/ethanol-based adhesive is used (Van Meerbeek *et al.*, 1996; Van Meerbeek *et al.*, 1998).

Self-Etch/ Etch-and-dry Approach

Regarding to SE adhesives, without the need of rinsing, the application time is shorter and the technique-sensivity lower (De Munck *et al.*, 2005a). Another important advantage of the self-etch approach is that infiltration of resin occurs simultaneously with the self-etching process, by which the risk of discrepancy between both processes is low or non-existent (Van Meerbeek *et al.*, 2003).

However, little is known about the long-term effects of incorporating dissolved hydroxyapatite crystals and residual smear layer remnants within the bond, and how much primer/adhesive solvent is kept within the interfacial structure should also be investigated (Van Meerbeek *et al.*, 2003). Such solvent will directly weaken the bond integrity, provide channels for nanoleakage or may affect polymerization of the infiltrated monomers. The resultant interfacial structure also becomes more hydrophilic

and, thus, more prone to hydrolytic degradation (Tay and Pashley, 2002).

Depending on etching aggressiveness, they can be subdivided into "strong", "mild" and "weak" self-etch adhesives.

"Strong" self-etch adhesives usually have a pH of 1 or lower, resulting in rather deep demineralization effects. At the enamel, the resulting etching pattern resembles a phosphoric-acid treatment following an etch&rinse approach (Pashley and Tay, 2001).

"Mild" self-etch systems, like Clearfil SE Bond, used in this study, in general, have a pH of around 2 and produce a partial superficial demineralization. The thickness of the hybrid layer is much thinner than that produced by the strong self-etch or E&R approachs, but thickness has been proven to be of less importance bonding effectiveness (De Munck *et al.*, 2003). The weakest property of mild self-etch adhesives is their bonding potential to enamel. In one study (Miyazaki *et al.*, 2000b), the bond strength decreased after thermo-cycling. Hence, developing monomers with stronger chemical bonding potential to hydroxyapatite may also help to further improve their bonding performance to enamel.

New adhesives, like Scotchbond universal, are classified as a one-step multi-mode adhesive system. Based on the special composition and according to manufacturer's instructions, the one-step multi-mode adhesive Scotchbond universal can bue used in the SE mode, as an E&R technique, or in selective enamel etching mode (de Goes *et al.*, 2014; Mena-Serrano *et al.*, 2013; Perdigao *et al.*, 2012). Nonetheless, there are few studies and clinical trials.

The etch-and-rinse approach remains the "gold standard" regarding the enamel bonding effectiveness when compared with SE adhesives (De Munck *et al.*, 2005a). This is because etching is relatively independent of smear-layer properties and preparation methods. The etching pattern created by self-etch adhesives, on the other hand, is less uniform, dependent on the acidity of the primer and smear-layer properties. Etching aggressiveness is, however, not entirely correlated with bonding effectiveness, as some mild and intermediary strong self-etch adhesives do approach the etch-and-rinse standard. This must be attributed to properties of the adhesive resin itself (Pashley and Tay, 2001).

The formation of taglike resin extensions into the enamel microporosities is considered the mechanism of bonding of resin to phosphoric acid-etched enamel (Miyazaki *et al.*, 2000b; Myers *et al.*, 1974). The resin-tag penetration into ground

enamel treated with self-etch adhesives has been deemed comparable to that of phosphoric acid-etch enamel (Gordan *et al.*, 1998), even though other studies reported that the etching pattern of self-etch adhesives was not as well-defined as that of total-etch adhesives both on ground enamel and on unground enamel (Pashley and Tay, 2001; Perdigao *et al.*, 1997; Tay *et al.*, 2004). In spite of a less defined etching pattern, self-etch adhesives may result in enamel bond strengths similar to those obtained after etching enamel with phosphoric acid (Ibarra *et al.*, 2002). However, other studies linked the lack of a defined etching pattern with low enamel strengths (Kanemura *et al.*, 1999).

It has been shown that enamel margins bonded with self-etch adhesives perform clinically at the same level as total-etch adhesives from 6 months to 2 years (Perdigao *et al.*, 2003; Turkun, 2003). But, besides the length of interprismatic enamel resin tags, several factors, such as the type and concentration of the acid, etching time, pH, proprietary formulation of the gels and acidic monomer, may influence the demineralization ability of acidic conditioners (Pashley and Tay, 2001; Zidan and Hill, 1986).

Bond Strength Testing

Under oral conditions, the interface between restoration and tooth is exposed to diverse forces that act simultaneously. The rationale behind bond strength testing is that the higher the actual bonding capacity of an adhesive, the better it will withstand such stresses and the longer the restoration will survive *in vivo* (Armstrong *et al.*, 2010).

Bond strength testing is relatively easy and fast, remaining the most popular methodology for measuring bonding effectiveness in the laboratory. The data obtained from bond strength tests largely depend on the actual test set-ups that may differ between laboratories for parameters such as specimen geometry, size of surface area, the type of composite and more (Pashley *et al.*, 1999). It is, therefore, not surprising that bond strength data substantially vary among laboratories throughout the world. There was also shown that microtensile bond strength was inversely related to the bonded surface area (Pashley *et al.*, 1999; Sano *et al.*, 1994).

The major disadvantage of µTBS-testing is the rather labor-intensive, technically demanding and relatively fragile sample preparation technique (Pashley *et al.*, 1999)

When bonding to enamel, an E&R approach still results in the highest bonding values. When pooling the µTBSs of all E&R adhesives tested (or of which the µTBS

was repeatedly measured as part of different studies), a µTBS of 39 and 40 MPa was achieved, respectively, for the three-step and two-step E&R adhesives. A SE procedure, in general, has resulted in a lower bonding effectiveness, though some adhesives approached the bonding effectiveness of E&R adhesives, with the average of 30 MPa. Statistical analysis of the pooled enamel µTBS data showed that E&R adhesives, irrespective of a two- or three-step application procedure, bonded slightly but significantly stronger to enamel than two-step SE adhesives and significantly more strong than one-step SE adhesives (Van Meerbeek *et al.*, 2003).

The objective of this study was to evaluate the effect of pre-etching on *in vitro* microtensile enamel bond strength of two adhesive systems: Universal Vs. Self-etch.

H_0:

There were no differences on the microtensile enamel bond strength values when comparing both adhesive systems: a universal and a self-etch adhesive.

2. Materials and Methods

2.1 Type of study

Experimental *in vitro* study with the purpose of evaluating the microtensile enamel bond strength of two adhesive systems: 1) Universal adhesive system as per manufacturer instructions used in etch-and-rinse mode, and 2) Self-etch adhesive with pre-etching phase, used in etch-and-rinse mode.

2.2 Design of the study

A convenient sample of eight 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 4ºC for one week and then, left in distilled water at 4ºC, according to the ISO TR 11405 standard (Standardization, 2003), no more than three months. All teeth were cleaned under running water using a periodontal scaler before preparation.

2.3 Teeth preparation

From each tooth, a crown segment was obtained 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 -; Fig. 1), on a hard tissue microtome (Isomet^{TM} 1000 Precision Saw, Buehler Ltd. Ltd., Lake Buff, IL, USA; Fig. 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 were attached to an acrylic holder with sticky wax, parallel to the long axis of the tooth (Fig. 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 cementoenamel junction to remove the roots (Fig. 4);
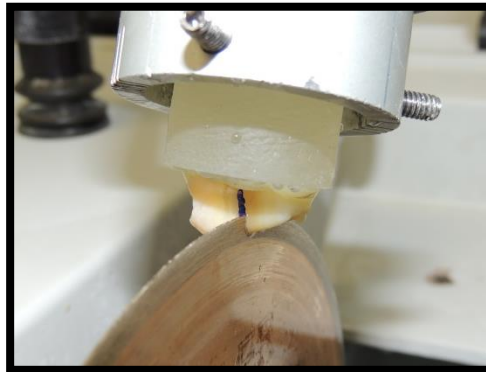

Figure 4 - Roots cutting 1-2 mm below cementoenamel junction.

3. The crowns were detached from the acrylic holders and the pulp tissues were removed from the pulp chamber with a dentin curette and diamond burs (Fig. 5, 6 and 7). The crowns were attached again to the acrylic holder with sticky wax (Fig. 8).

Figure 5, 6 and 7 – Removal of the pulp tissues.
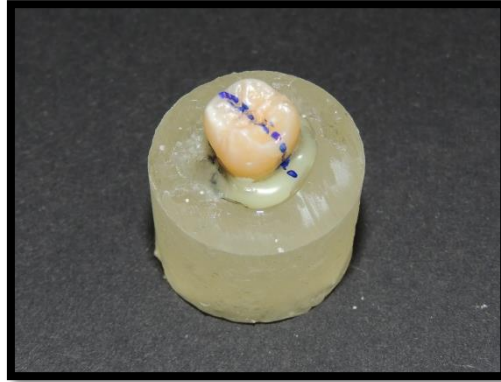
Figure 8 – The crown fixed to the acrylic holder.

3.   The crowns were sectioned mesiodistally, parallel to the long axis of tooth, using a diamond disk at low speed, under constant water irrigation (Fig. 9), obtaining two enamel surfaces, a vestibular and a lingual/palatal.
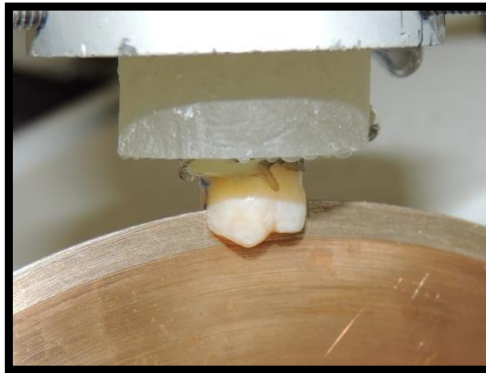
Figure 9 – Mesiodistal crown cutting.

4.   The pulp chambers of the two pieces were then filled with cyanoacrylate glue (737 Black Magic Toughened adhesive, Permabond, Hampshire, UK) and the crown segments were fixed with the same glue to the acrylic holders, by the pulpal side (Fig. 10);

Figure 10 – Crowns glued with cyanoacrylate glue to acrylic holders.

5. With the purpose of creating a uniform smear layer obtained in similar conditions to those occurring in clinical situations, the enamel surface was cut with a cilindric high-speed diamond bur during 5 seconds under water irrigation (Pashley *et al.*, 1988).

2.4 Distribution and treatment of the crown segments

The crown segments were kept in distilled water until the moment of treatment. 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.

The adhesion area was marked in the flattest area of the segments surface. The occlusal thirds of the vestibular and lingual surfaces were discharged due to their slope.

All the treatment procedures were performed by the same operator in the way that is followed described:

Group A – Scotchbond Universal Adhesive (3M ESPE Seefeld, Germany) as per manufacturer's instructions – Total-etch (Etch-and-Rinse) technique on enamel (SBU TE E):

1. The enamel surface was slightly dried, without desiccate, but kept moist using a moist cotton pellet, so that the surface remained shiny and visibly moist.
2. The 32% phosphoric acid etching gel (Scotchbond Universal Etchant; 3M ESPE Seefeld, Germany) was applied to the marked enamel surface, during 15 seconds, and then rinsed with water and air-dried without desiccate. The excess of water was removed using a moist cotton pellet, so that the surface remained shiny and visibly moist.
3. The adhesive was applied, using a disposable microbrush, to the marked enamel surface, scrubbing lightly for 20 seconds.
4. 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.

5.   Finally, the surface was polymerized for 10 seconds.

Figure 11 – Scotchbond Universal Adhesive.

Group B – Clearfil SE Bond Adhesive (Kuraray, Okayama, Japan) with pre-etching – Total-etch (Etch-and-Rinse) technique on enamel (CL TE E)*:

* - It is a modification of manufacturer´s instructions. Not recommended by manufacturer.

1.   The enamel surface was slightly dried, without desiccate, but kept moist using a moist cotton pellet, so that the surface remained shiny and visibly moist.

6.   The 32% phosphoric acid etching gel (Scotchbond Universal Etchant; 3M ESPE Seefeld, Germany) was applied to the marked enamel surface, during 15 seconds, and then rinsed with water and air-dried without desiccate. The excess of water was removed using a moist cotton pellet, so that the surface remained shiny and visibly moist.

2.   The primer was applied, using a disposable microbrush, to the marked enamel surface, and left in place for 20 seconds.

3.   The adhesive was applied, using a disposable microbrush, to the marked enamel surface, scrubbing lightly for 20 seconds.

4.   The surface was then gently air-dried, 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.

5. The adhesive was applied using a disposable microbrush, leaving a uniform, thin and even adhesive layer, removing the excess of adhesive with the same microbrush and with a soft blow of air.

6. Finally, the surface was polymerized for 10 seconds.

Figure 12 – Clearfil SE Bond plus the Scotchbond Universal etchant.

The adhesive polymerizes with visible light, so it was used a device with a lid to block natural light, and it was used at maximum of 3 minutes after placing it in the dispenser.

Resin composite build-ups were performed using a resin composite, ENAMEL Plus HRi (Micerium S.p.A. Avegno (GE) Italy; Fig. 13), 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 (Fig. 14). 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/cm^2 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/cm^2 with a radiometer (Curing Radiometer P/N 10503, USA) throughout the procedure.
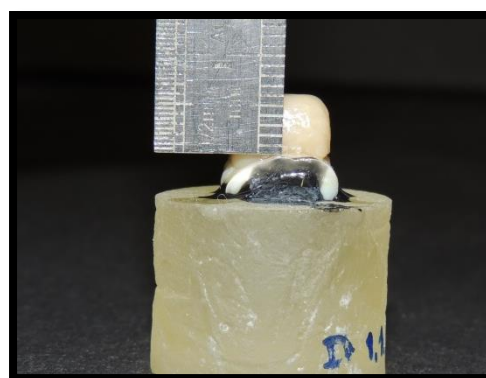
Figure 13 – Resin composite ENAMEL Plus HRi

Figure 14 – Resin composite build-up.

Material	Manufacturer	Components	Lot code and expiring date
Clearfil SE Bond Adhesive	Kuraray, Okayama, Japan	**PRIMER:** 10-MDP; HEMA; Hydrophobic aliphatic dimethacrylate; dl-Camphorquinone; Water; Accelerators; Dyes; Others. **BOND:** 10-MDP; Bis-GMA; HEMA; Hydrophobic aliphatic methacrylate; dl-Camphorquinone; Colloidal silica; Initiators; Accelerators; Others.	Primer Lot code: 2U0022 Expiring date: 07-2015 Bond: Lot code: 2T0039 Expiring date: 07-2015
Scotchbond Universal	3M ESPE Seefeld, Germany	Bis-GMA; HEMA; (10-MDP) Decamethylene dimethacrylate; Ethanol; Water; Silane treated silica; 2-propenoic acid, 2-methyl-, Reaction products with 1,10-Decanediol and phosphorous oxide (p2o5); Copolymer of acrylic and itaconic acid; Dimethylaminobenzoat(-4); Camphorquinone (dimethylamino)ethyl methacrylate; Methyl ethyl ketone.	Lot code: 490251 Expiring date: 09-2014
Ortophosphoric acid 32 %	Scotchbond Universal Etchant; 3M ESPE Seefeld, Germany	Ortophosphoric acid (H_3PO_4) 32%; Thickener; colorant; deionized water	Lot code: 537103 Expiring date: 11-2015
Enamel plus HRi Composite UD4	Micerium S.p.A Avegno (GE) Italy	Dymethachrylates; barium glass; Iteberium Trifluorethum; mixed oxides; pre-polymers; aditives; catalysts; stabilizers; pigments; 1,4-Butandioldimethacrylate; Urethandimethacrylate (UDMA); Bis-GMA	Lot code: 2012 000 921 Expiring date: 12-2018

Table 1 – Manufacturer, components and lot code of the adhesives, acid and composite used

2.5. Specimens preparation for the microtensile tests

All teeth were painted with different colors with waterproof ink. The exterior surface of the resin composite was also painted, in order to identify them.

The teeth were stored in distilled water in an incubator for 24 hours at 37 °C. Date and time of the restoration was registered.

Posteriorly, the teeth were longitudinally sectioned in both "x" and "y" (Fig. 15 and 16) directions with a slow-speed diamond disk (Diamond Wafering Blade - 10,2cmx0,3mm - Series 15HC, Buehler Ltd., Lake Bluff, IL, USA) under water irrigation, using a hard tissues microtome (Isomet^{TM} Precision Saw, Buehler Ltd. Ltd., Lake Buff, IL, EUA), to obtain sticks with a cross-sectional area of approximately 1 mm^2.

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.
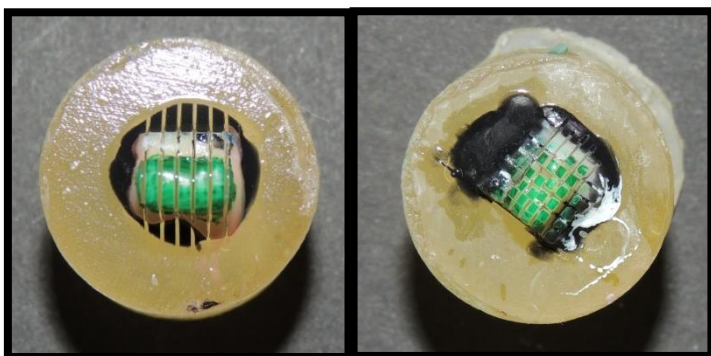
Figure 15 and 16 – The teeth after being sectioned in both "x" and "y" directions.

Figure 17 – The sticks obtained after the final cut.

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

The obtained sticks (Fig. 17) were kept in distilled water for a maximum of 24 hours, until the conclusion of the microtensile tests.

Group	Tooth	Sticks obtained	Debonded sticks	Lost sticks	Sticks tested	Sticks tested per group
A	1	20	0	0	20	58
	2	14	0	1	13	
	3	18	2	2	14	
	4	11	0	0	11	
B	1	18	1	4	13	61
	2	18	0	1	17	
	3	17	0	0	17	
	4	15	0	1	14	
Total		131	3	9	119	119

Table 2 – Characterization of sticks obtained, per group and per tooth.

2.6. Microtensile tests

The specimens were individually attached to a stainless-steel grooved Geraldelli´s jig (Fig. 18 and 20) with cyanoacrylate glue (737 Black Magic Toughened adhesive, Permabond, Hampshire, UK) and then submitted to a tension load using a universal testing machine (Instron 4502 H 3307, Norwood, MA, USA; - Fig. 19), at a crosshead speed of 1mm/min until fracture occurred.

Figure 18 – Specimens attached to Geraldelli's jig.

Figure 19 – Instron 4502, universal testing machine.

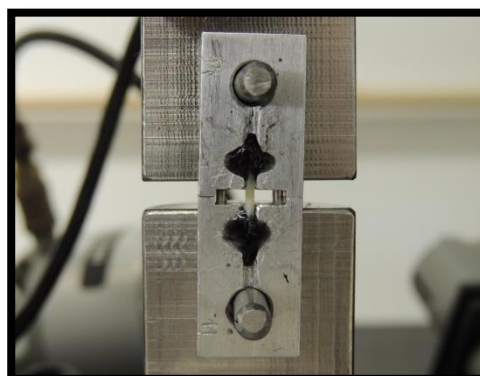*Figure 20 – The specimen attached to a Geraldelli's jig.*

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 specimen were registered and µTBS calculated in MPa, by dividing the force imposed at time of fracture by the bond area (mm^2).

The failure modes were analyzed under a stereomicroscope at 10x and classified as adhesive (A) - failure occurring at the enamel-adhesive interface; cohesive when the failure occurred in enamel (CD); or in composite (CC); and mixed (M) - failure with composite and enamel/dentin at the interface.

2.7. Statistical Analysis

The statistical analysis of the results was performed through descriptive and inference methods.

The statistical analysis with the Kolmogorov-Smirnov test determined that $p>0,05$ (0,200) so we could use a parametric test. Also the Levene's test for equality of variances determined that $p>0,05$ (0,383), so the samples/groups are homogenous, allowing the t-test interpretation be done in safe.

A paired-sample t-test was performed since the assumption of normality and equal variances among groups were valid.

Pretesting failures that occurred during specimen preparation were treated as left-censored data (Armstrong *et al.*, 2010).

3. Results

The number of microtensile beams (N), means, and standard deviations (SD) are shown in table 3. Clearfil SE (CL TE E) resulted in similar µTBS means (24,73 ± 13,38 MPa) in comparison to Scotchbond Universal (SBU TE E) (24,45 ± 10,88 MPa).

Variable =MPa

Group	N	Minimum	Maximum	Mean	Standard Deviation	Std error Mean
A - SBU TE E	58	3,90	45,36	24,4517	10,87830	1,42839
B - CL TE E	61	1,31	82,01	24,7379	13,37784	1,71286

Table 3 – Mean and SD of µTBS (MPa) per group.

In this graphic is shown the µTBS values for the two experimental groups.
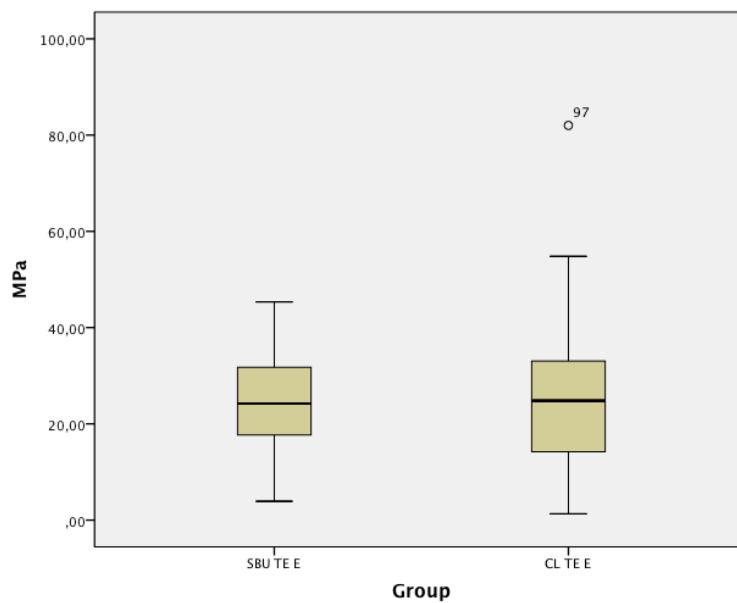
Graphic 1 – Distribution of the µTBS (MPa) values for the two experimental groups.

There were not statistically significant differences among the two groups as revealed by the t-test ($p < 0,05$).

|		t-test for Equality of Means			
		t	**df**	**Sig. (2-tailed)**	**Mean Difference**
MPa	**Equal variances assumed**	-,128	117	,899	-,28627
	Equal variances not assumed	-,128	114,288	,898	-,28627

Table 4 – t-test

Table 5 and graphic 2 summarizes the types of failures obtained: in number and in percentage, per experimental group. 39,50% of the fractures were adhesive, without significative differences between groups, A (39,93%) and B (40,98%).

Type of failure										
Group	**Adhesive**		**Mixed**		**Cohesive composite**		**Cohesive enamel/ dentine**		**Total**	
	N	%	N	%	N	%	N	%	N	%
Group A - SBU TE E	22	37,93	21	36,21	5	8,62	10	17,24	58	100
Group B - CL TE E	25	40,98	16	26,23	6	9,84	14	22,95	61	100
Total	47	39,50	37	31,09	11	9,24	24	20,17	119	100

Table 5 – Characterization of the different types of failures per group.
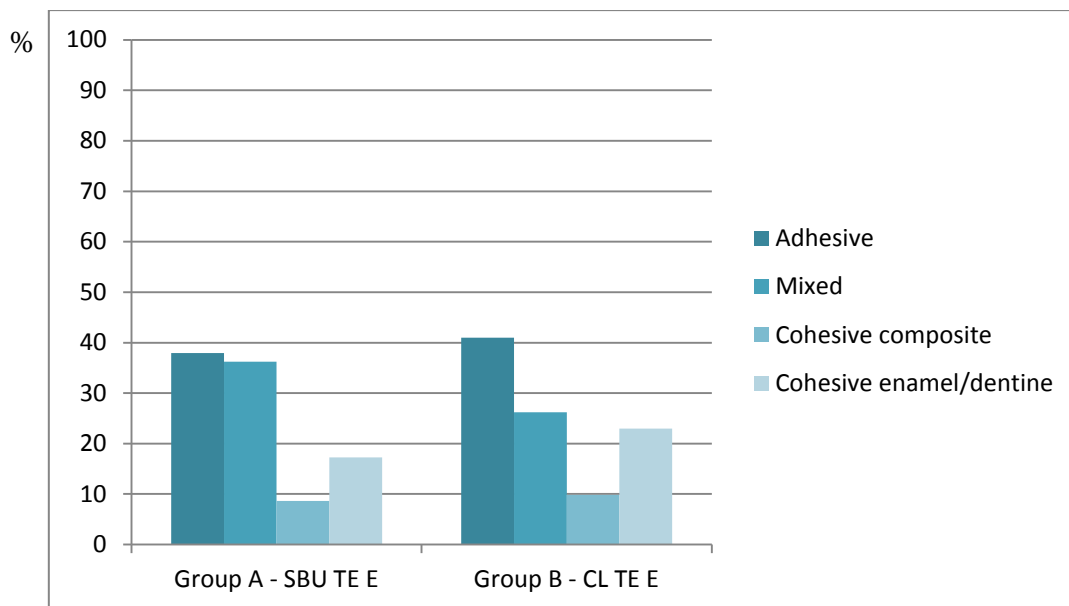
Graphic 2 – Characterization of the different types of failures per group.

4. Discussion

The clinical success of the adhesive restorations depends, not only, on the correct manipulation of the adhesive system, but also on the mode of application. There are differences between the self-etch mode application and the total-etch (Peumans *et al.*, 2005), although self-etching systems have undergone a rapid evolution over the past few years. It becomes necessary to test the new adhesives introduced in the market, like Universal adhesives, and to assess if alterations in their mode of application change their bond strength.

The aim of this study was to analyse the microtensile bond strength of two adhesive systems, a universal and a self-etch one, and find out if the new universal adhesive system produced similar values to a self-etch adhesive widely studied.

After the experimental work and statistic analysis of the data, was possible to accept the null hypothesis and affirm, due to the obtained results, that there are no differences when comparing µTBS values of the universal and the self-etch adhesive.

µTBS test is one of the most used to study the most recent adhesive systems launched by diverse manufacturers (Armstrong *et al.*, 2010), and for that reason was the method used in this study.

Clearfil SE Bond is now considered the "gold standard" for all SE adhesives. In the laboratory, CSE tended to provide high dentin and enamel bond strengths (Brackett *et al.*, 2008; Burrow *et al.*, 2008; De Munck *et al.*, 2005a; Mine *et al.*, 2009; Perdigão *et al.*, 2008; Van Landuyt *et al.*, 2006; Van Landuyt *et al.*, 2009). Likewise, an excellent clinical performance of CSE, with a 93 and 100% retention rate, respectively, at 3 years was reported in two studies (Turkun, 2003; Van Meerbeek *et al.*, 2004). And one study (Peumans *et al.*, 2010) resulted in a 98% retention rate at 8 years with or without separate enamel etching, another study (van Dijken, 2010) reported a retention rate on a yearly basis up to 8 years for CSE as compared with a 2-step etch-and-rinse adhesive. Also, a 5-year clinical study (Peumans *et al.*, 2007) compared restorations bonded with CSE using recommended procedures compared with selective etching of the enamel margins with phosphoric acid. The results found that 36% of the restorations with recommended procedures had no enamel defects compared with 66% of the restorations having no defects when selective etching was used.

These two different adhesive systems were selected because the Universal

adhesive (Scotchbond Universal) studied is very recent and insufficiently studied, at least on enamel. The universal adhesive was study on total-etch mode and the self-etch with a manufacturer's modification, introducing a pre-etch phase.

The bond strength of these adhesive systems showed non-significant bond strength. These results are in agreement with data from a recent similar study (Freitas *et al.*, 2012). So it seems that the bond strengths values are equal, when using a self-etch adhesive system or a universal one, on enamel. This was expected since both adhesives contain similar components, like 10-MDP, a functional phosphate monomer.

The presence of the molecule 10-MDP, which has chemical affinity for dental tissues (Yoshida *et al.*, 2004), leads to an exposure of calcium and phosphate binding sites (Fukegawa *et al.*, 2006), which are necessary for adhesion and to which the monomer binds in the second step. On enamel, the hydroxyapatite crystals are larger than in dentin, and the reactive surface is smaller, therefore acids have to be stronger to produce demineralization. MDP when interacting with hydroxyapatite forms a stable nano-layering at the adhesive interface (Inoue *et al.*, 2005; Yoshida *et al.*, 2012; Yoshihara *et al.*, 2010). The hydrolysis of the ester bond from the acidic monomer results in a strong phosphoric acid that might increase the demineralization over time (Wang and Spencer, 2005).

Also SBU has polyalkenoic acid copolymer, besides MDP, in its formulation. Although both molecules may compete by binding to the calcium in hydroxyapatite (Yoshida *et al.*, 2012), and they are usually associated with improved adhesive performance (Perdigao *et al.*, 2013; Van Meerbeek *et al.*, 2011), and that might be why the SBU had similar µTBS to CSE.

Besides acid concentration of acid used on the pre-etching, other factor that may play a role in the magnitude of enamel bond strengths are the orientation of the enamel prisms and the cohesive strength of the adhesive resin (Carvalho *et al.*, 2000; Pashley and Tay, 2001). That is why in this study the enamel surfaces used where flattened parallel to the tooth axis, to standardize the orientation of enamel prisms.

Also, all adhesives tested were applied to bur-cut smear-layers prepared using high-speed diamond burs, used commonly for adhesive cavity preparation at the clinic. This procedure resulted in a uniform smear-layer with a roughness comparable to a smear layer created with SiC paper (Mine *et al.*, 2010; Wahle and Wendt, 1993). Besides standardized bur-cut smear-layer formation, literature concerning the technique

sensivity of the bonding procedure demonstrates that the performance of adhesive systems is significantly influenced by the technique variability of the operators, in particular procedures like etching, rinsing and drying (Giachetti *et al.*, 2007; Giachetti *et al.*, 2008; Miyazaki *et al.*, 2000a). Therefore, all the bonding procedures in this study where made by the same operator.

Even though the manufacturer´s instructions are well-formulated, they are not very detailed and have some degree of ambiguity. For that reason an effort an effort was made to specify each step as much as possible, so that the protocol would become more consistent and standardized.

The specimen geometry has a significant influence on the homogeneity of stress concentration, which should be minimized. Due to the lack of consensus on specimen design and the relatively higher incidence of pre-testing failures during trimming, the stick-shaped specimens are simpler to prepare when compare to a dumbbell and hourglass geometry, and seem to introduce less flaws and stress during specimens preparation, so in this study was chosen to use stick/beam-like specimens (Armstrong *et al.*, 2010; Ghassemieh, 2008; Pashley *et al.*, 1999). Nonetheless, the stick shaped type of specimens present an higher standard deviation than the hourglass shaped ones (Ghassemieh, 2008).

In the present study the intensity all the light curing was controlled by a radiometer (Bala *et al.*, 2005) to ensure that the intensity of the curing light was equal during all experimental work. Halogen light was used, since the literature indicates better results with this type of light (Pereira S., 2000).

Results of microtensile bond strength tests cannot easily be translated to clinical effectiveness of adhesive systems. Under intra-oral conditions, restorative interfaces are susceptible to degradation by saliva and other factors.

There are additional limitations when trying to extrapolate the *in vitro* results to the clinical performance of a material. An example of this, is the storage in water 24 hours before the testing, to simulate the intra-oral conditions (Huysmans *et al.*, 1996). Another point observed is the high standard deviation obtained in all experimental work. Multiple imperfections may have occurred during the experimental part of the study. Bubbles or failures in the adhesive layer, lack of uniformity in the thickness of the adhesive, angulated traction forces (stick glued in the jig with a slight deviation, or not perfectly parallel to the Geraldelli's jig, or problems standardizing the moisture on

enamel, are all preponderant factors that may yield huge variations in standard deviation of the microtensile force values (Ghassemieh, 2008; Perdigao *et al.*, 2002)3

From a clinical point of view, etching with phosphoric acid is problematic. Selective etching on enamel would be necessary, since etching dentine reduces the bond strength (Torii *et al.*, 2002; Van Landuyt *et al.*, 2006; Walter *et al.*, 2011). Pronounced etching or over-etching of dentine leads to a total and deep demineralization of the collagenous structures, which have to be infiltrated by the adhesive system. Selective etching without affecting the dentine, however, is very difficult to achieve in the daily clinical practice.

5. Conclusions

The results of the present study require the acceptance of the null hypothesis that there are no differences on the µTBS values when comparing a universal and a self-etch adhesive. Based on these results, it seems that for enamel, multi-mode universal adhesives yield similar adhesive performance than the gold standard two-step self-etching adhesives when the latest are also applied with selective enamel pre-etching. Further clinical studies are needed with these newest multi-mode universal adhesives, like Scotchbond Universal, especially because these materials are being increasingly used on patients without proof of clinical efficacy.

The need of selective etching on enamel is highlighted by the generally lower bond strengths found for SE systems to enamel compared with E&R systems (De Munck *et al.*, 2003; De Munck *et al.*, 2005b; Goracci *et al.*, 2004; Loguercio *et al.*, 2008). Selective etching without affecting dentine, however, is very difficult in daily clinical practice. Hence, a pre-treatment not leading to over-etching dentine but maintaining bond strength to both dentine and enamel would be desirable.

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7. Appendix

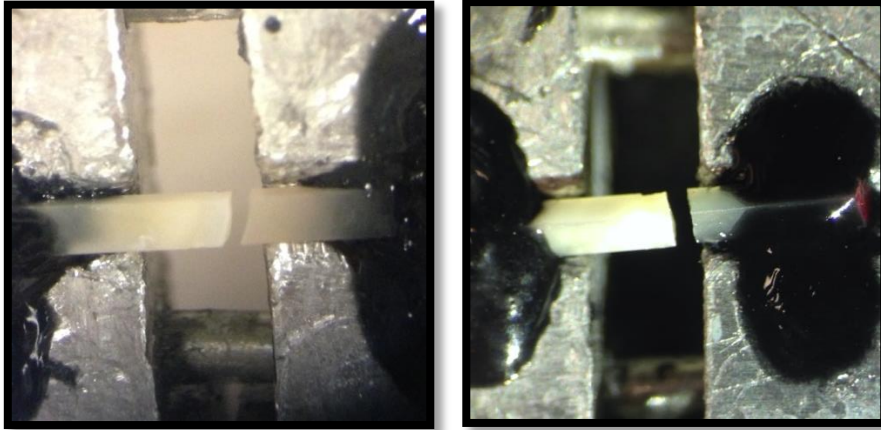**I – Stereomicroscopy photographs of the different types of failures:**

A) Adhesive failures

Figure I and II – Adhesive Failures.
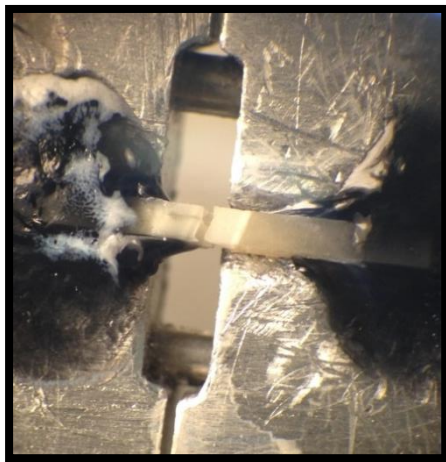
B) Cohesive failures

a. Enamel-Dentine

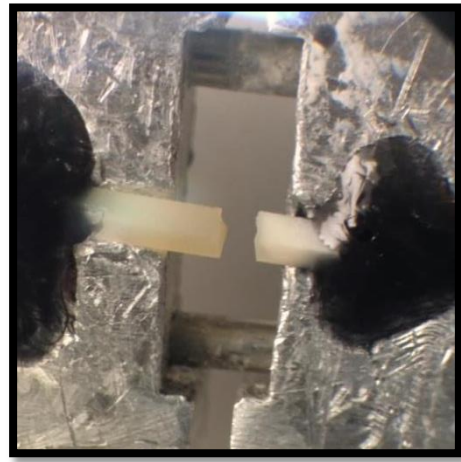b. Composite

Figure III: Cohesive failure – Enamel-Dentine.

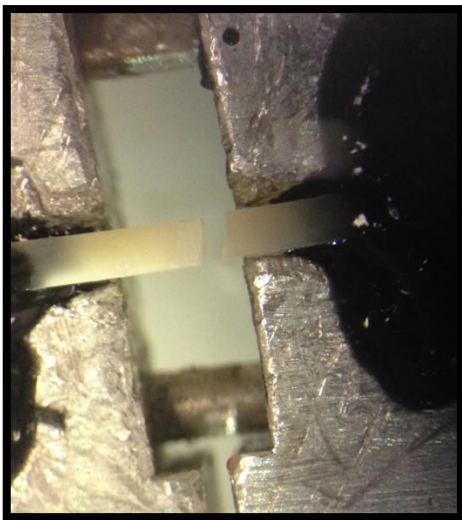*Figure IV: Cohesive failure – Composite.*

C) Mixed failures.

Figure V: Mixed failure.

II – Tables

| Group		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
MPa	SBU TE E	,054	58	,200[*]	,979	58	,423
	CL TE E	,089	61	,200[*]	,911	61	,000

*. This is a lower bound of the true significance.

*Table I: Shapiro-Wilk and kilmogorov.Smirnov normality tests*

		t-test for Equality of Means		
		Std. Error Difference	95% Confidence Interval of the Difference	
			Lower	Upper
MPa	Equal variances assumed	2,24188	-4,72619	4,15366
	Equal variances not assumed	2,23029	-4,70433	4,13180

Table II: t-test

III - Graphics
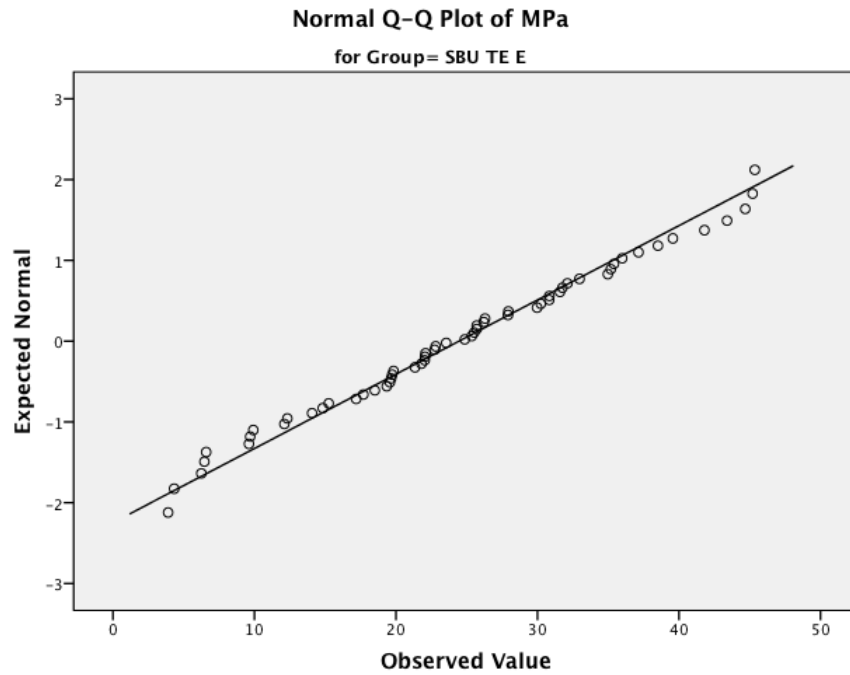
Graphic I: Normal distribution of the values for Group A: Scotchbond Universal Adhesive (3M ESPE Seefeld, Germany)on enamel
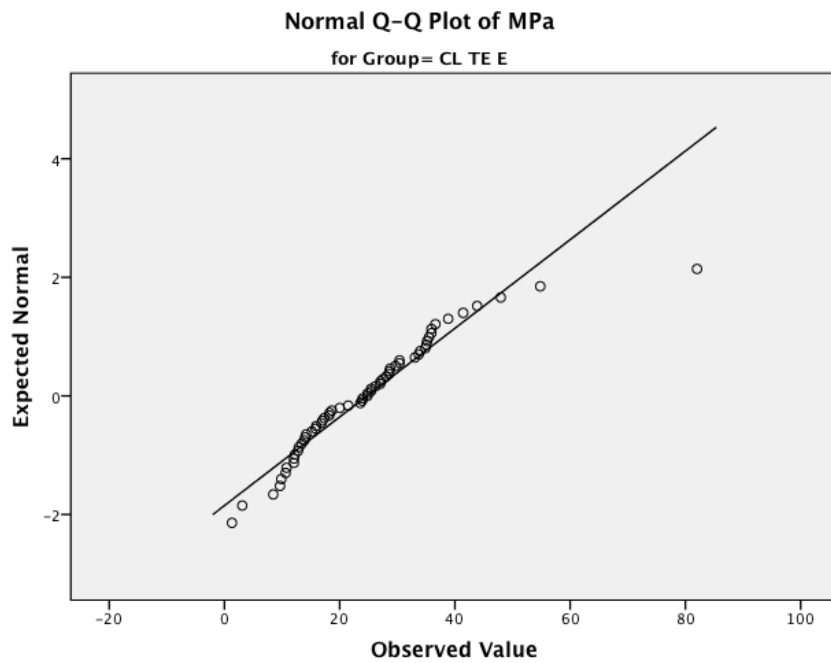
Graphic II: Normal distribution of the values for Group B: Clearfil SE Bond Adhesive (Kuraray, Okayama, Japan)on enamel

IV – Manufacturer's instructions

Scotchbond Universal Adhesive (3M ESPE Seefeld, Germany) as per manufacturer's instructions:

1. Selective Enamel Etching
   a. Apply a commonly used phosphoric acid etching gel (about 35%), e.g., Scotchbond Universal Etchant to the prepared and unprepared (if present) tooth enamel and allow to react for 15 sec.
   b. Rinse thoroughly with water and dry with water-free and oil-free air or with cotton pellets; do not overdry.
2. Total Etching Procedure
   a. 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 sec.
   b. Rinse thoroughly with water and dry with water-free and oil-free air or with cotton pellets; do not overdry.
3. Use the disposable applicator to apply the adhesive to the entire tooth structure and rub it in for 20 sec. Avoid contact between the adhesive and the oral mucosa.
4. Subsequently direct a gentle stream of air over the liquid for about 5 sec until it no longer moves and the solvent has evaporated completely.
5. Harden the adhesive with a commonly used curing light for 10 sec.

Clearfil SE Bond Adhesive (Kuraray, Okayama, Japan) as per manufacturer's instructions:

1. Dispense the necessary amount of primer into a well of the mixing dish immediately before application
2. Apply primer to the entire cavity wall with a sponge or a disposable brush tip. Leave it in place for 20 seconds.
3. After conditioning the tooth surface for 20 seconds, evaporate the volatile ingredients with a mild oil-free air stream.

4. Dispense the necessary amount of bond into a well of the mixing dish.

5. Apply bond to the entire surface of the cavity with a sponge or a disposable brush tip.

6. After application, make the bond film as uniform as possible using a gentle oil-free air stream.

7. Light-cure the bond for 10 seconds with a visible light curing activator.