IN VITRO ANALYSIS OF BOND STRENGTH OF SELF-ETCHING ADHESIVES APPLIED ON SUPERFICIAL AND DEEP DENTIN

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ABSTRACT

The purpose of this study was to evaluate the bond strength of three adhesive systems to superficial and deep dentine using the microtensile bond strength test (µTBS).

The occlusal enamel of thirty human third molars was removed to expose a flat surface of superficial or deep dentin. For each type of surface, the test specimens were randomly divided into three groups which underwent the application of a conventional two-step adhesive system [Single Bond (SB)] as the control group (n=10), a two-bottle self-etching system [One Coat SE Bond (OCSE)] (n=10) and a one bottle one-step system [Clearfil S^3 Bond (CFS^3)] (n=10). Adhesives were applied, a 5-mm high "crown" as built-up with resin composite Z250 (3M) and the specimens with a cross-sectional area of 0.7 ± 0.1 mm² were tested in tension (0.5 mm/min). Four fractured sticks from each tooth were randomly selected and the dentin side was gently abraded with a 1200-grit SiC paper, etched with 35% phosphoric acid for 15 s and air dried. SEM micrographs at 70X and 2400X magnification were taken using scanning electron microscopy (SEM) to calculate the area of tubular dentin (ATD) and tubular density (TD) with Image Pro Plus 5. Two-way ANOVA (dentin depth-adhesive) showed higher bond strength values for SB. However, the values did not depend on dentin depth. Linear regression showed a significant relationship between bond strength and area of intertubular dentin for SB (p=0.004), and a significant inverse relationship between tubular density and bond strength for CFS^3 (p=0.009). OCSE exhibited a tendency that was similar to SB and opposite to CFS^3, but was not statistically significant. The conventional two-step adhesive had higher bond strength values. The use of digital image analysis facilitates the manipulation of data and contributes to the interpretation of the behavior of new adhesive systems.

Key words: tensile strength, dentin-bonding agents, dentin, scanning electron microscopy.

ANÁLISIS *IN VITRO* DE LA RESISTENCIA DE UNIÓN DE ADHESIVOS AUTO-CONDICIONANTES APLICADOS SOBRE DENTINA SUPERFICIAL Y PROFUNDA

RESUMEN

El objetivo de este trabajo fue evaluar la resistencia de unión de tres sistemas adhesivos aplicados sobre dentina superficial y profunda por medio del ensayo de microtracción.

Se obtuvieron superficies planas de dentina superficial y profunda por medio del desgaste del esmalte oclusal de treinta terceros molares humanos. Para cada de superficie, los especímenes fueron separados aleatoriamente en tres grupos según el tipo de sistema adhesivo utilizado: convencional de dos pasos [Single Bond (SB)] como grupo control (n=10), auto-condicionante de dos pasos [One Coat SE Bond (OCSE)] (n=10) y un sistema adhesivo auto-condicionante de paso único [Clearfil S^3 Bond (CFS^3)] (n=10). Después de su aplicación, se reconstruyó la porción coronaria a una altura de 5 mm con resina compuesta Z250 (3M). Se obtuvieron especimenes por medio de cortes seriados perpendiculares entre si con un área de sección transversal de 0.7 ± 0.1 mm², los cuales fueron sometidos al ensayo de microtracción (0.5mm/min). Se seleccionaron aleatoriamente cuatro especimenes fracturados de cada diente y el lado dentinario fue desgastado con lija de carburo de silicio de granulación 1200, acondicionados con ácido fosfórico 35% por 15 s y secados al aire. Para el cálculo del área de dentina tubular (ADT) y densidad tubular (DT) con el programa Image Pro Plus 5 se tomaron fotomicrografías con MEB en aumentos de 70X y 2400X. Si bien un ANOVA de dos vías (profundidad dentinaria-adhesivo) mostró valores superiores de resistencia de unión para SB, estos valores no fueron condicionados por la profundidad de la dentina. La prueba de regresión linear mostró una relación significativa entre resistencia de unión y área de dentina intertubular para SB (p=0.004), y una relación inversa significativa entre densidad tubular y resistencia de unión para CFS^3 (p=0.009). OCSE presentó una tendencia similar a SB y opuesta a CFS^3, sin embargo, sin significación estadística.

El sistema adhesivo convencional de dos pasos tuvo valores superiores de resistencia adhesiva; y la utilización del análisis digital de imágenes facilitó el manejo de lo datos y una mejor interpretación del comportamiento de los nuevos sistemas adhesivos.

Palabras clave: resistencia a la tracción, agentes de recubrimiento dental adhesivo, dentina, microscopia electrónica de barrido.

INTRODUCTION

Recent advances in dentin adhesion allow present dental treatments to be less invasive and demand a continuous investigation of the bonding mechanism of newly developed adhesives. The acid-etch technique employing 30-40% phosphoric acid resulted in the typical pattern of microporosities that provide substrate for micromechanical retention[1]. Although

Table 1: Batch number, composition and application procedures of adhesive systems

Materials	Composition (batch number)	Application mode
Adper Single Bond (3M ESPE, St Paul, USA)	*Etchant:* 35% phosphoric acid gel. (6GK) *Adhesive:* dimethacrylates, HEMA, polyalkeniod acid copolymer, 5 nm silane treated colloidal silica, ethanol, water, photoinitiator. (4JU)	*a, b, c, d, f, g, h*
One Coat SE Bond (Coltène/Whaleden, Altstätten, Switzerland)	*Primer:* water, HEMA, acrylamidosulfonic acid, glycerol mono- and dimethacrylate, methacrylized polyalkenoate *Bonding:* HEMA, glycerol mono- and dimethacrylate, UDMA, methacrylized polyalkenoate, CQ. (NL692)	*d, e, f, g, h*
Clearfil S^3 Bond	MDP, Bis-GMA, HEMA, photo-initiators, ethanol, water, silanated colloidal silica. (00070A)	*d, f, g, h*

a) acid-etching; b) rinsing; c) air-drying; d) dentin kept dry or rewetted with water; e) apply primer; f) apply adhesive; g) air-dry for solvent evaporation; h) light-curing.
HEMA: 2-hydroxyethyl methacrylate; Bis-GMA: bisphenol A diglycidyl methacrylate; UDMA: urethane dimethacrylate; MDP: 10-methacryloyloxydecyl dihydrogenphosphate; CQ: camphorquinone.

the success of the etching-bonding technique on enamel has been largely proven, the bonding on dentin is different due to the morphology and composition of the substrate[2]. In an effort to overcome these problems, self-etching adhesives were developed mainly to overcome postoperative tooth sensibility[3]. Although long-term evaluation of self-etching adhesives was deficient, current research suggests that their dentin bond strength and bonding stability were comparable to that of total-etch adhesives[4].

To achieve an optimal dentin bond, the adhesive must create micromechanical retention by resin infiltration of the demineralized dentin and penetration in tubules and branches before polymerization[5]. The relative contribution of resin tags to bond strength will depend on where the bond is made (superficial, middle, or deep dentin) and whether the dentinal tubules are perpendicular or parallel to the prepared dentin surface.[6]

The heterogeneous structure of dentin that varies both in composition and microstructure, not only between teeth but along the same teeth, creates difficult conditions for adhesion, contributing to the low reliability of many restorations. In the same way, this variability of dentin as substrate for bonding generates controversial results on the behavior of bonds when adhesives are applied on superficial or deep dentin.

Therefore, profiting from the advantages of the microtensile test, which permits small bonded surface areas to be evaluated, and the use of an automated procedure for systematic classification of the dentin as a function of the depth, the purpose of this study was to evaluate quantitatively the effect of dentin depth on bond strength of two self-etching adhesives and correlate these values (MPa) with intertubular dentin area (IDA) and tubule density (TD) calculated by digital image analysis.

MATERIALS AND METHODS

Thirty extracted, caries-free human third molars were used. The teeth were collected after obtaining the patient's informed consent under a protocol approved by the University Estadual of Ponta Grossa Institutional Review Board. A flat dentin surface was exposed after wet grinding the occlusal enamel on a # 180 grit SiC paper. The exposed dentin surfaces were further polished on wet # 600-grit silicon-carbide paper for 60 s to standardize the smear layer.

The adhesives examined were: the two-step etch&rise adhesive Adper Single Bond (SB - 3M ESPE, St. Paul, MN, USA), the two-step self-etching adhesive One Coat Self Etching Bond (OCSE – Coltène/Whaledent, Altstätten, Switzerland) and the one-step self-etch adhesive Clearfil S^3 Bond (Kuraray Medical Inc, Tokyo, Japan). The composition, application mode and batch number are described in Table 1.

The surfaces were then rewetted with water, the adhesives were applied on surfaces according to the manufacturer's directions and light-cured using a quartz-tungsten halogen light set at 600 mW/cm^2 (Optilux, Demetron Research Corporation, USA) confirmed by a curing radiometer (Model 100, Demetron Research Corporation, USA). Resin composite build-ups (Z250, shade B2, 3M ESPE, St. Paul, MN, USA) were constructed on the bonded surfaces in increments. Each one was individually light-cured for 20 s with the same light intensity.

After storage in distilled water at 37ºC for 24 h, each tooth was sectioned with a saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) through the composite buildups and dentin at 0.8 mm increments, in both mesio-to-distal and buccal-to-lingual directions, to produce beams with a cross-sectional area of approximately 0.7 mm^2.

Each beam to be tested was fixed to a specific device for microtensile testing with cyanoacrylate resin and pulled to failure under tension using the Micro Tensile Tester (Bisco, Schaumburg, IL, USA) at a crosshead speed of 0.5 mm per minute. The failure modes were evaluated at 40X with a microscope (Eclipse E200, Nikon, USA) and classified as: (C) cohesive, if failure occurred exclusively within dentin (CD) or resin composite (CR); (A) adhesive, if failure occurred at the resin/dentin interface; (A/M) adhesive/mixed, if failure occurred at the resin/dentin interface and included cohesive failure of the neighboring substrates.

A systematic procedure based on digital image analysis was developed to quantify tubular density and the area occupied by tubules. Initially, the dentin side of fractured specimens with adhesive failure was treated according to the technique reported by Giannini et al.[7] (Fig. 1A-C). Photomicrographs at 70X and at 2400X from representative areas were taken. Micrographs at higher magnification were digitally modified with the software *Adobe Photoshop 7.0* (Fig. 1D) to calculate the density of tubules and the area occupied by tubules for each specimen with the *Image Pro Plus 5* software (Fig. 1E-F).

Microtensile bond strength (μTBS) data obtained for all the subgroups were analyzed by two-way ANOVA to examine the effects of adhesive systems and dentin depth. Multiple comparisons were done using Tukey's test. Statistical significance was set at the 0.05 probability level. Liner regression analyses were done to correlate individual bond strength, tubule density and area occupied by tubules.

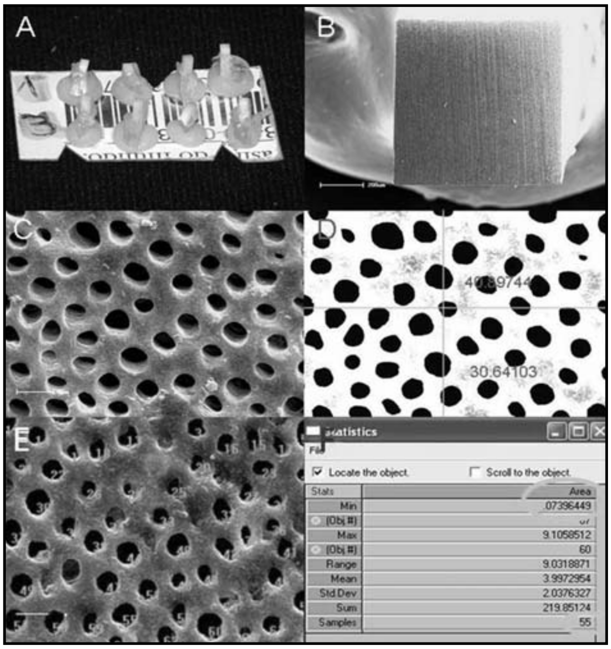

Fig. 1: Digital image analysis to calculate tubule density and intertubular dentin area. A) Beams positioned on plastic support; B) Photomicrograph at 70X; C) Photomicrograph at 2400X; D) Image modified by Photoshop 7.0; and measurement of total area of the image; E) Measurement of tubule density and area occupied by dentin tubules; F) Original values obtained by Image Pro Plus 5.

RESULTS

The mean μTBS and standard deviations for the different adhesive systems are summarized in Table 2. Two-way ANOVA, confirmed by Tukey's test showed that significant differences were observed only for the factor dentin adhesives ($p < 0.05$). Single Bond showed higher μTBS values, both in superficial and deep dentin, than One Coat SE Bond and Clearfil S^3 Bond. Bond strengths for OCSE and CFS^3 were statistically similar, and independent of preclassified dentin depth.

Table 2: Microtensile bond strength (Mean±SD) for each experimental group

Dentin depth	*Adper Single Bond*	*One Coat SE Bond*	*Clearfil S3 Bond*
Superficial	53.36 (±5.85)Aa	34.23 (±6.8)Bb	34.95 (±7.26)Bb
Deep	55.71 (±8.84)Aa	24.17 (±3.09)Bb	29.60 (±3.60)Bb

Same upper and lower-case letters indicate no significant differences (p>0.05).

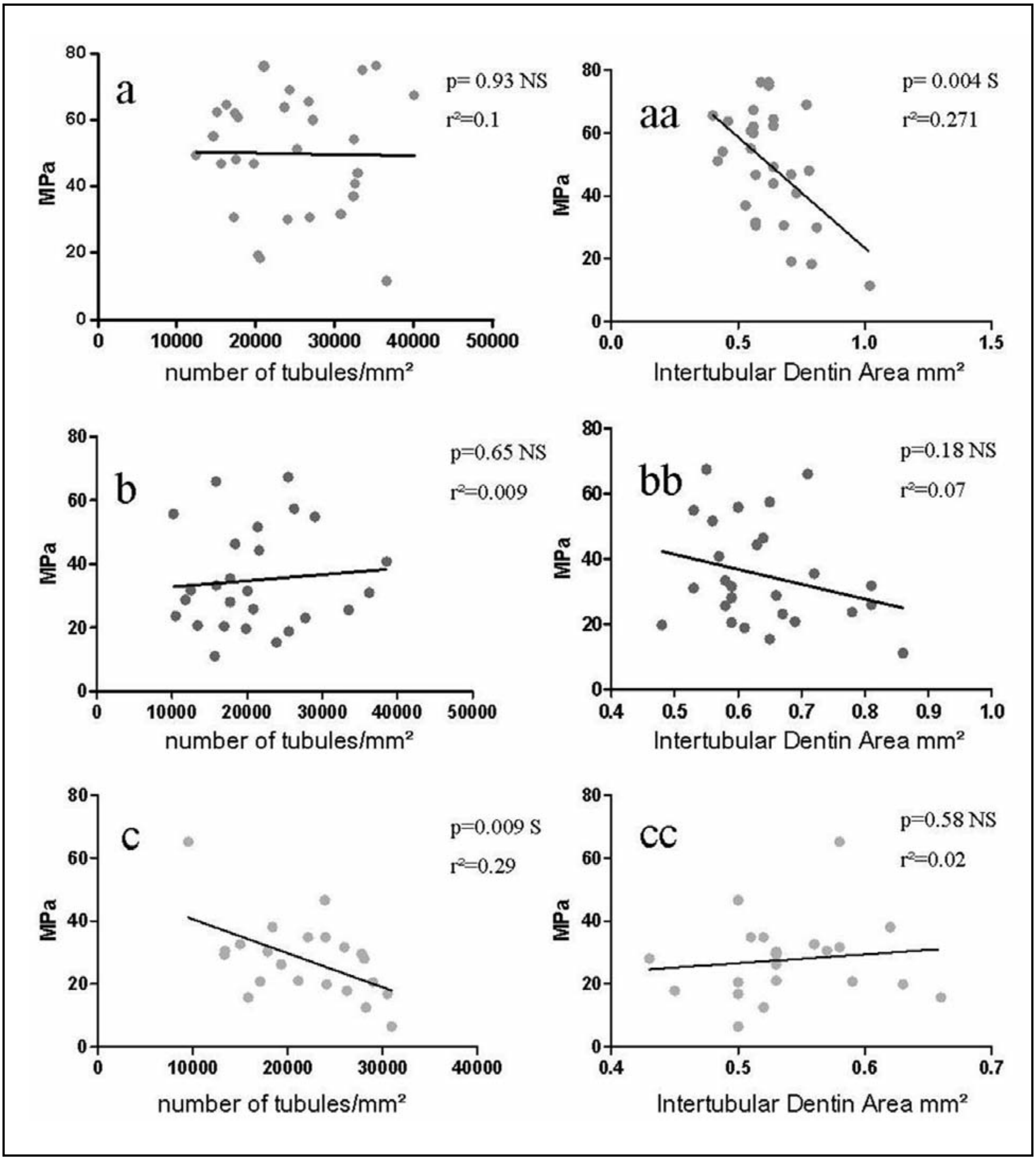

Fig. 2: Pearson correlation values for Single Bond (a, aa), One Coat SE Bond (b, bb) and Clearfil S[3] Bond (c, cc).

Linear regression analysis of the correlation between individual bond strength values and intertubular dentin area (IDA) (Figure 2 aa-bb) revealed that the behavior of Single Bond and One Coat SE Bond was similar, exhibiting a tendency to a reduction in bond strength as the IDA increased. This trend was statistically significant only for SB (p=0.004). CFS[3] showed an inverse relationship compared to SB and OCSE. However the difference did not reach statistical significance (Fig. 2c-cc).

DISCUSSION

New adhesive systems continue to be launched. However, the differences found in their behavior in relation to dentin depth, demand new studies on the mechanisms of formation of the hybrid layer and bond strength to dentine[5, 7-14].

Although laboratory studies cannot reproduce all the variables involved in oral conditions, they offer an alternative to understand the behavior of the adhesive systems that are developed continuously, undergoing rapid evolution and modifications in their chemical formula[15]. The microtensile method (µTBS) has advantages such as more adhesive failures, the possibility of measuring higher interfacial bond strength values, and since very small bonded areas are used, it allows for the measurement of regional bond strength variations within the same teeth, facilitating the SEM examination of the failed bonds[16,17]. Most of the studies that evaluate the µTBS of dentin employ methods such as trimming or non-trimming tests, measuring dentin depth in terms of the Remaining Dentin Thickness (RDT), obtained with a digital caliper from pulp chamber to the dentinal surface prepared with a diamond saw[9,18-23]. However, RDT can not be measured in all bonded sticks due to the angle of the section with the pulp chamber[9,24,25]. This fact can influence the outcome of the test itself and the procedure employed to attach the microspecimen to the µTBS jig bonded sticks[26]. However, recent studies have demonstrated that the number and diameter of dentin tubules vary in form and composition along the dentin thickness, and even from point to point on a given tooth.[13, 27, 28]

Within this context, Giannini et al.[7] correlated the bond strength values with the tubule density and the area occupied by solid dentin at the site of fracture. As in another studies[20], they calculated the tubule density and the average diameter of tubules by hand by direct counting on each micrograph. The lack of consensus on the behavior of adhesive systems related to dentin depth might be due to the use of inappropriate methodologies which preclude a more accurate analysis on the influence of regional variations of dentin. To overcome such interpretation drawbacks of the method, this study recommends a systematic procedure to classify the dentin as a function of the shape and density of dentin tubules based on digital image analysis, disregarding the preclassification of dentin depth as a function of transversal sections of the crowns. This concept becomes evident when the tubular density (22.572±7.338 tubules/mm^2) and the area occupied by tubules (15,67±7,06%) are considered globally as the substrate for the three adhesives. These values would be more representative of a superficial and middle dentine than of deep dentin, in agreement with Eick et al.[18].

As reported by Kenshima et al.[30], the etch-&-rinse system presented a markedly thicker hybrid layer and produced a higher density of tags with a more uniform distribution along the whole dentin surface than the self-etch systems. These differences would be related to the acidity of adhesive systems, solvent concentration, adhesive conversion, cohesive strength of the adhesive system, hybridization efficiency, substrate variations and operator-related factors. The new adhesive system Clearfil S^3 Bond has the same component as Clearfil SE Bond, one of the most reliable adhesive systems[31]; the 10-methacryloxydecyl dihydrogen phosphate (MDP) which might explain the reported high bond strength results of this mild one bottle self-etch system, similar to that obtained by One Coat SE Bond. Although Single Bond obtained higher bond strength values, the specimens were submitted to the microtensile bond strength test immediately after 24 h. As already mentioned, self-etching adhesives are less susceptible to degradation over time due to their capacity to fully infiltrate the demineralized dentin[32,33].

CONCLUSIONS

Within the limits of this study, it may be concluded that the two-step etch&rise adhesive Adper Single Bond produced statistically higher microtensile bond strength values, irrespective of preclassification of dentin depth. New methodologies such as systematic digital image analysis will facilitate a better understanding of the behavior of adhesive systems.

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