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Influence of Additional Adhesive Application on the Microtensile Bond Strength of Adhesive Systems

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Clinical Relevance

Applying an additional layer of solvent-free adhesive systems increased adhesive thickness and did not affect bond strength. This thicker adhesive layer may help to preserve the integrity of the restoration by acting as a stress-absorbing layer.

SUMMARY

This study evaluated microtensile bond strength (µTBS) when an additional adhesive layer was applied to the dentin surface. Thirty-five human third molars were flattened to expose the occlusal dentin surface. The teeth were randomly assigned to 7 experimental groups: G1—Single Bond (SB); G2—additional layer of SB; G3—a layer of Scotchbond Multi-purpose (SMP) adhesive applied over SB; G4—Clearfil SE Bond (CE); G5—additional layer of CE; G6—Adper Prompt (AP) and G7—additional layer of AP. For the G2, G3, G5 and G7 groups, the first adhesive layer was light-cured before application of the additional layer. After bonding procedures, 5-mm high composite crowns were incrementally built up. The samples were sectioned to obtain 0.9 x 0.9 beams, which were tested under tension at a crosshead speed of 0.5-mm/minute until failure. The failure mode and adhesive thickness were evaluated under SEM. The µTBS data were analyzed by 1-way ANOVA and post-hoc Ducan's Test (α=0.05). Mean adhesive thickness was analyzed by 1-way ANOVA and post-hoc Tukey's test (α=0.05). The results indicated that G3 presented the highest µTBS and the thickest adhesive layer. G6 and G7 presented the lowest µTBS values. When solvent-free adhesives systems were used,

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µTBS values were not affected by the thicker layer.

INTRODUCTION

During resin composite polymerization, the intermolecular space occupied by Van Der Walls forces undergoes changes and is replaced by shorter covalent bond spaces.[1] This modification results in resin composite shrinkage, which, when surrounded by bonded cavity walls, generates stresses over the adhesive interface.[2] If high bond strength values are obtained, such as those between the adhesive system and tooth enamel, the polymerization shrinkage stress created is transferred to the dental structure, and this, added to the enamel elastic modulus, will result in cracks evidenced by well defined white lines.[3-4]

The dentin substrate, due to its heterogenic and dynamic characteristics, undergoes a more complex adhesive process, resulting in lower bond strength values. This means that rupture of the adhesive layer can result in gaps at the restoration margin.[5-6] Dentin acid etching exposes collagen fibers surrounded by polymerized monomer resin; this area, known as the hybrid layer, has a relatively low elastic modulus.[7-8] As the component overlying the hybrid layer, the adhesive layer may help to preserve the integrity of the hybridized dentin, protect it from polymerization shrinkage stresses and act as a stress absorbing layer.[9] However, the adhesive layer thickness is not enough to act as a stress absorbing layer.[10]

In order to create a relatively thick intermediate layer with low elastic modulus between dentin and composite with the objective of absorbing shrinkage stresses through an elastic deformation, one option is to apply a second adhesive layer.[1,11-12] With this in mind, Zheng and others[13] showed that, for simplified adhesive systems that present solvents in their composition, the improved adhesive thickness makes it more difficult to volatilize the solvent before photopolymerization, resulting in decreased bonding values. The solution to this problem could be to photopolymerize each layer before a subsequent application.

Therefore, this study evaluated the effect on the microtensile bond strength of applying an additional adhesive layer at the tooth-restoration interface after photopolymerizing the first layer. In addition, the adhesive layer thickness was measured by scanning electron microscopy to evaluate the correlation between adhesive thickness and µTBS at the dentin-adhesive interface.

METHODS AND MATERIALS

Thirty-five human third molars, stored in 0.05% thymol saline solution for a maximum of 3 months, were used to conduct this study. Each tooth was sectioned perpendicular to its longitudinal axis, using a slow-speed diamond saw under water coolant, to expose mid-coronal dentin. Each surface was ground with 600-grit Silicon Carbide paper under running water for 15 seconds just before bonding. The teeth were separated into 7 experimental groups—5 teeth each—according to the adhesive procedure to be used. Table 1 lists the components and manufacturers used.

The bonding procedures were carried out as follows:

Group 1—The dentin surface was acid-etched with phosphoric acid for 15 seconds, then thoroughly washed, using water spray, for 15 seconds. Excess water was removed with a wet cotton pellet,[14] and Single Bond adhesive was applied in 2 consecutive layers (manufacturer's instructions). After 30 seconds, the solvent was gently evaporated for 3 seconds with directed low-pressure air stream and was photopolymerized for 10 seconds.

Table 1: *Materials Used in This Study*

Materials	Mains Components	Manufacturer
3M Scotchbond Etchant	35% phosphoric acid, colloidal silica	3M ESPE, St Paul, MN, USA
Single Bond (SB)	HEMA, Bis-GMA, PAA, CQ, Ethanol, water	3M ESPE, St Paul, MN, USA
Scotchbond Multi-purpose Adhesive (SMP)	Bis-GMA, HEMA	3M ESPE, St Paul, MN, USA
Clearfil SE Bond (CE)		Kuraray Co Ltd, Osaka, Japan
Primer	10-MDP, HEMA, hydrophilic dimethacrylate, N,N-diethanol p-toluidine, water	
Adhesive	10-MDP,Bis-GMA, HEMA, hydrophobic dimethacrylate, CQ, N,N-diethanol p-toluidine, silanated colloidal silica	
Adper Prompt (AP)		3M ESPE, St Paul, MN, USA
Liquid A	Methacrylated phosphoric esters Bis-GMA, Initiators based on camphorquinone	
Liquid B	Stabilizers Water, HEMA, Polyalkenoic acid, Stabilizers	
Spectrum TPH	Urethane modified Bis-GMA, silanated Ba-Al-B-silicate glass, CQ, EDAB	Dentsply/De Trey, Konstanz, Germany

Abbreviations: 10-MDP: 10-Methacryloyloxydecyl dihydrogen phosphate; HEMA: 2-hydroxyethyl methacrylate; PAA: polyalkenoic acid copolymer; Bis-GMA: bisphenol-glycidyl methacrylate; CQ: dl-canforquinone.

Group 2—After adhesive application and photopolymerization in the same manner as in Group 1, 2 more consecutive layers of the same adhesive were applied and gently air dried before photopolymerization for 10 seconds.

Group 3—After adhesive application and photopolymerization in the same manner as Group 1, a layer of Scotchbond Multi-Purpose adhesive was applied and photopolymerized for 10 seconds.

Group 4—The self-etching primer of the Clearfil SE Bond adhesive system was applied to the dentin surface, left undisturbed for 20 seconds and evaporated using an air syringe. The CE adhesive was then applied, spread gently with the air syringe and photopolymerized for 10 seconds.

Group 5—After adhesive application and photopolymerization in the same manner as in Group 4, more consecutive layers of the same adhesive were applied, the CE adhesive was applied and it was photopolymerized for 10 seconds.

Group 6—One drop each of Adper Prompt adhesive system liquids A and B were mixed and applied to the dentin surface and agitated for 15 seconds. An additional layer was applied and, after gentle air-jetting, was photopolymerized for 10 seconds.

Group 7—After adhesive application and photopolymerization in the same manner as in Group 6, the AP system was mixed and applied again in 2 consecutive layers (as recommended by the manufacturer), air-jetted and photopolymerized for 10 seconds.

Following the bonding procedures, five 1-mm increments of a resin composite were built up and individually photopolymerized for 20 seconds. After the restorative procedures, the samples were stored in distilled water at 37°C for 24 hours.

Microtensile Bond Strength Test

The samples were sectioned perpendicular to the adhesive interface into 0.9-mm thick slabs using a slow-speed diamond saw under water-cooling. The slabs were sectioned again in a perpendicular direction to get approximately 0.8-mm^2 beams. One beam from each tooth was separated for the adhesive layer thickness measurement, while the other beams were submitted to microtensile tests.

The beams were attached to the flat grips of a microtensile testing device using cyanocrylate cement and were tested under tension in a Universal Testing Machine, with a crosshead speed of 0.5 mm/minute, until failure. After testing, the specimens were removed from the fixtures and the cross-sectional area of the fracture sites were measured with a digital caliper to calculate the ultimate tensile bond strength expressed in MPa. The means of each tooth were calculated and the data analyzed using 1-way ANOVA. The Duncan adjustment for multiple comparisons was preformed at a 5% of level of significance.

Mode of Failure Analysis

After microtensile testing, the dentin sides of fractured specimens were mounted on aluminum stubs, gold-sputter coated and observed by SEM at 200x or higher magnification for fracture mode determination. Failure modes were classified to be among 4 different types:

Type 1– adhesive failure between adhesive and dentin.

Type 2– partial adhesive and partial cohesive failure in adhesive.

Type 3– partial cohesive failure in dentin.

Type 4– total cohesive failure in adhesive or failure between the adhesive and composite.

Adhesive Layer Thickness Measurements

The selected beams, prepared above, were observed by SEM for adhesive layer thickness measurement. A 1000-grit SiC paper was used to remove defects and angles from each sample under water. The beams were then polished using different diamond paste granulations (6, 3 and 1 µm) and a polishing cloth under mineral oil cooling. The interface sections were rinsed with water, while polishing debris and paste were ultrasonically removed for 5 minutes. The specimens were demineralized with a 50% (w/v) H_3PO_4 acid solution for 3 seconds, then immersed in a 1% NaOCl solution for 10 minutes to remove the non-encapsulated collagen fibrils. Each beam was gold-sputter coated and observed by SEM. The thickness of the adhesive layer was measured directly on the microscope monitor using a multi-point measuring device. Three different measurements were made per beam, and the mean obtained was used for statistical analyses. The data were analyzed by 1-way ANOVA and post hoc Duncan's test at a 5% level of significance. The results of the microtensile bond strength were correlated with adhesive thickness through Pearson correlation.

RESULTS

The microtensile results are shown in Table 2. Group 3 presented the highest µTBS means and revealed statistical differences when compared to the other groups. Group 1 presents the second highest µTBS means. Groups 6 and 7 presented the lowest values but showed no statistical difference between them (*p*=0.78). Groups 2, 4 and 5 presented intermediate values that were lower than those of Groups 3 and 1, yet higher than those of Groups 6 and 7.

The failure mode for each group is summarized in Figure 1. Groups 1 and 4 presented more type 2 failures. All the other groups showed a greater number of type 4 failures, which represented 53%, 66%, 73%, 90% and 76% of the total failures for Groups 2, 3, 5, 6 and 7, respectively.

The experimental group thickness measurements are summarized in Table 3. Group 3 showed the highest mean adhesive thickness value, demonstrating statistical difference compared with the other groups. Group 5 presented the second highest thickness, followed by Group 2. Groups 7 and 4 showed no statistical difference between them (p=0.98) and were superior to Groups 1 and 6, which presented the lowest adhesive thickness values. The Pearson correlation between microtensile bond strength and adhesive layer thickness was not significant (r=0.16, p=0.34).

DISCUSSION

As a composite inserted into a cavity shrinks, stresses are created and transferred to the adhesive interface.[11] The incidence of these forces is correlated to the composite deformation capacity, thus dissipating the stress created. Consequently, when there is a reduction in adhered surface, there is also an increase in composite deformation during contraction. In this study, a plain adhesive surface was used, implying that there was only one adhered face. Such polymerization stress was minimal, due to the unconstrained adhesive resin flow during polymerization. Therefore, only the bond strength given by the adhesive system and the influence of the additional adhesive application were evaluated, since the forces over the adhesive interface were practically restricted to tensions created during application of the microtensile test.

When comparing the adhesive systems that were applied according to the manufacturers' recommendations, the bond strength of SB was higher than CE, and the AP system presented the lowest values. The hybrid layer is considered to have the most important role in dentin adhesion. Therefore, the bonding agent must penetrate through the collagen system of etched dentin and be effectively photopolymerized to generate high bond strength values. The adhesive system AP is classified as "All-in-one," meaning that its concentration of organic solvents is high, since it needs to dissolve the resin components and ionize the acidic resin monomer responsible for its self-etching characteristic. Evaporation of these solvents reduces the thickness of the adhesive layer. Zheng and others[13] showed that adhesive layers thinner than 25 µm are not adequately

Table 2: *Results of Microtensile Bond Strength Test*

Groups	Means in MPa (SD)	n
Group 3	38.36 (5.51)^{a}	5
Group 1	29.76 (4.36)^{b}	5
Group 4	24.86 (3.88)^{c}	5
Group 5	23.93 (1.55)^{c}	5
Group 2	21.70 (1.94)^{c}	5
Group 7	13.83 (2.22)^{d}	5
Group 6	11.98 (1.23)^{d}	5

Same superscript letters indicate no statistical difference (α=0.05)

Table 3: *Results of Adhesive Layer Thickness Measurements*

Groups	Means in µm (SD)	N
Group 3	419.97 (21.91)^{a}	5
Group 5	179.21 (6.70)^{b}	5
Group 2	146.72 (7.28)^{c}	5
Group 7	69.51 (5.99)^{d}	5
Group 4	66.37 (6.97)^{d}	5
Group 1	34.51 (4.86)^{e}	5
Group 6	19.64 (1.42)^{f}	5

Same superscript letters indicate no statistical difference (α=0.05)

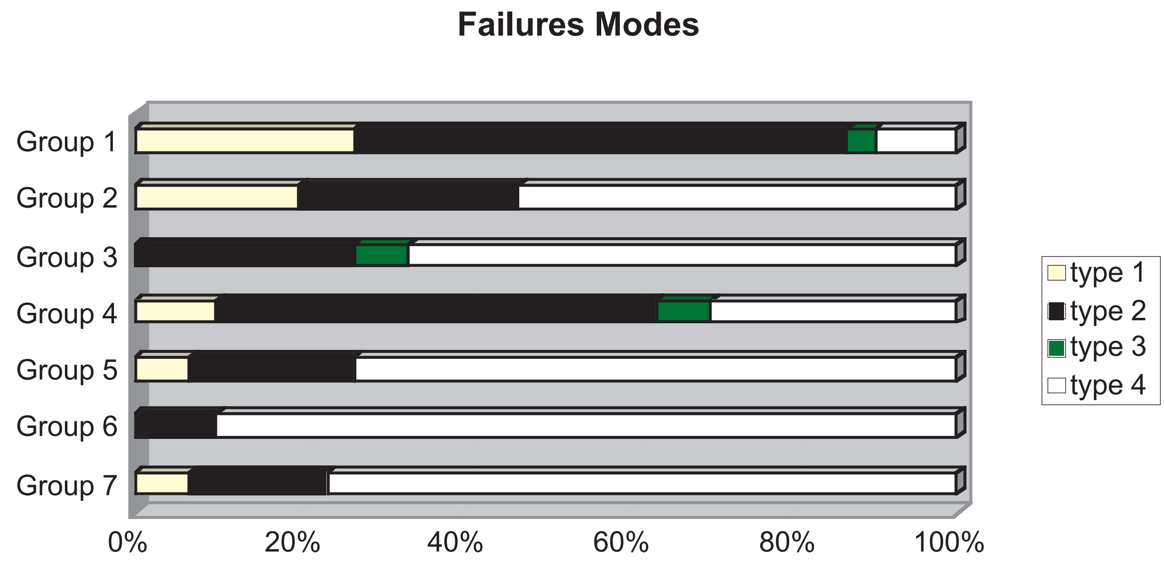

Figure 1. *Graphic illustrating the incidence of the different modes of failure.*

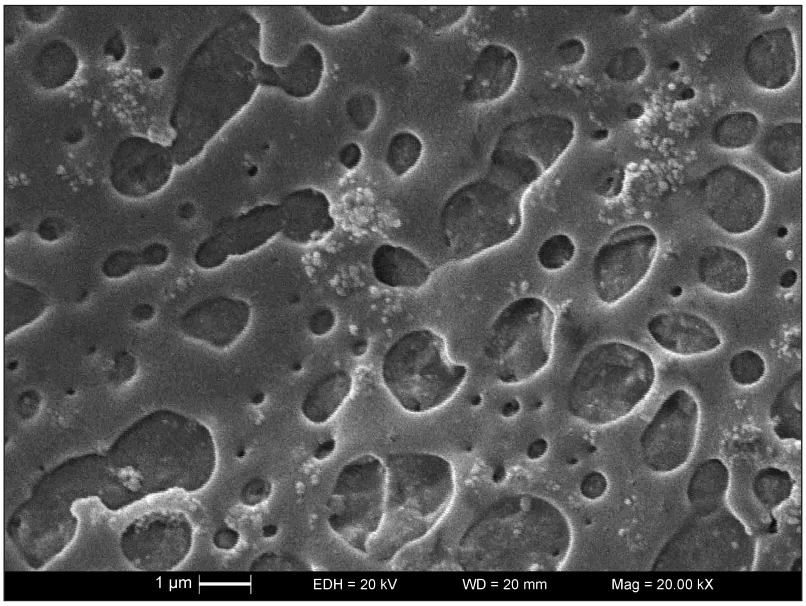

Figure 2: *Adper Prompt fractured surface after microtensile testing. Note the type 4 failure mode and the presence of bubbles in the adhesive.*

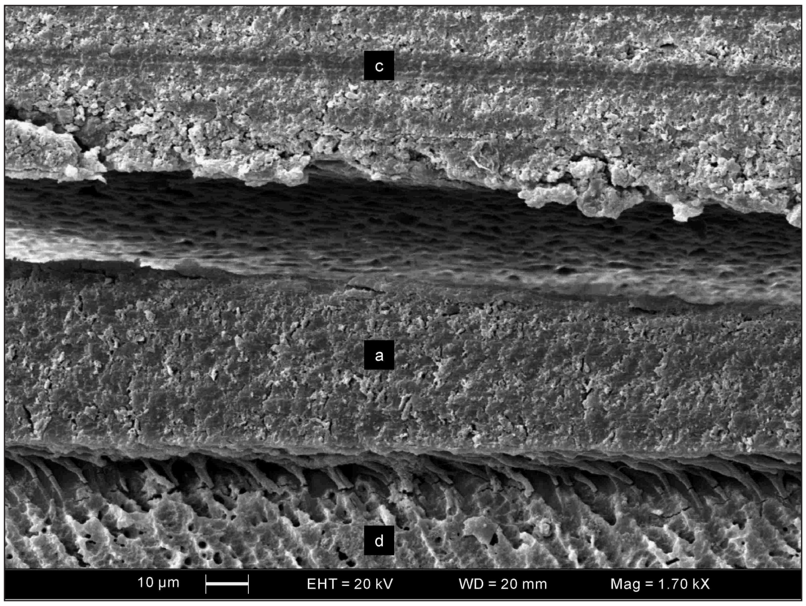

Figure 3: *Adhesive interface of Group 6 (conventional application of Adper Prompt adhesive system). Note the gap between the composite and adhesive and the presence of bubbles on top of the adhesive layer and fractured tags. c: composite; a: adhesive; d: dentin.*

polymerized due to the inhibition caused by oxygen. Pashley and others[15] found higher μTBS for AP when a second adhesive layer was applied, followed by polymerization of the first layer. The authors observed that an additional application of the bonding agent could seal the non-polymerized layer oxygen, thus enabling it to be adequately polymerized. However, in this study, the additional adhesive application did not increase the μTBS values.

Moreover, the oxygen inhibition layer and the presence of water in the adhesive may compromise the polymerization reaction and create pores through the adhesive layer.[16-17] The short 10-second application time is not long enough to allow for complete water elimination. Water is the solvent used in the AP system and presents low volatility. In addition, there were dissolved calcium and phosphorus ions present in the "all-in-one" adhesives as a result of etching of the dental substrate. These ions osmotically attract water, which diffuses through the adhesive layer to create a water blister.[18] The porosities inside the adhesive layer, which can be observed in Figures 2 and 3, could act as initial sites for early flaws during the microtensile test.[19]

Figure 2 shows disrupted blisters in the adhesive layer of a sample from Group 6 after microtensile testing. As the adhesive interface shows in Figure 3, it is possible to identify blisters in the top portion of this layer, and a gap between the adhesive and composite can be seen. Since the specimens were not fixed prior to SEM observation, it is conceivable that these gaps may have originated from or may have been increased by desiccating the specimens for SEM observation. However, since no gaps were observed in the other adhesive system groups, and since all specimens were treated equally, the differences are attributed to the lowest bond strength of the AP system. Pashley and others[15] observed an undulated surface at the base of the composite layer in the specimens bonded with Prompt L-pop (a previous version of AP), suggesting that the composite did not intermix with the top portion of the unpolymerized adhesive monomers. Bubbles may also be observed in Figure 3.

The results for bonding agent SB showed that a second application decreased bond strength values (Group 2). In this study, 2 additional layers were applied after photopolymerization of the first; they increased the adhesive thickness from a mean of 34.51 μm to 146.72 μm (Figure 4), showing a difference greater than 100 μm. According to Zheng and others,[13] thicker layers of adhesive resins might present a lower degree of conversion than thinner layers, which might explain the lower bond strength means for Group 2. They found a reduction in microtensile bond strength and an increase in cohesive failures for SB when there was a layer thicker than 100 μm. According to the authors of the current research, this may be explained because the 2 different solvents of SB (ethanol and water) present different volatilities. When these solvents are in a same solution, ethanol evaporates faster until it reaches its azeotrope, when both ethanol and water present the same speed of volatilization. Therefore, in thin layers, these 2 solvents may easily be volatilized. On the other hand, in thicker layers, ethanol evaporates even faster. In this case, the volume of ethanol lost prior to reaching its azeotrope may permit some resin monomers to fall out of the solution, causing phase separations within the adhesive layer. This, added to the difficulty of evaporating the adhesive's residual water, could lead to a low degree of adhesive conversion and decreased physical properties.

The application of SMP adhesive after SB photopolymerization revealed high µTBS. SMP adhesive does not present solvents in its composition, resulting in a thicker adhesive layer.[13,20] According to Ausiello, Apicella and Davidson,[21] the greater the adhesive thickness, the higher the elastic effect, since the stress difference is transformed into adhesive layer deformation. This adhesive layer thickness increase of around 419.97 µm may have been able to absorb and distribute the tensile tensions generated during the test. Therefore, this same adhesive layer may act inside a cavity preparation to reduce the composite polymerization shrinkage stresses. Furthermore, this increased adhesive layer may reduce stress created by thermal variations and mechanical loading and will probably improve the longevity of the restoration.

The additional application of the CE adhesive did not change the µTBS values. As in the SMP system, the CE adhesive has no organic solvents in its composition. In addition to a thicker adhesive layer, its bonding agent curing is easier, because there is no need for solvent volatilization. Figure 5 illustrates the thickness of the adhesive layer for the CE adhesive system and the improvement obtained when the second layer was applied. However, results showed that the µTBS values were not influenced. In addition, the difference between thicknesses presented by Groups 4 and 5 was not as great as that evidenced by Groups 1 and 3 (SB with and without SMP adhesive application).

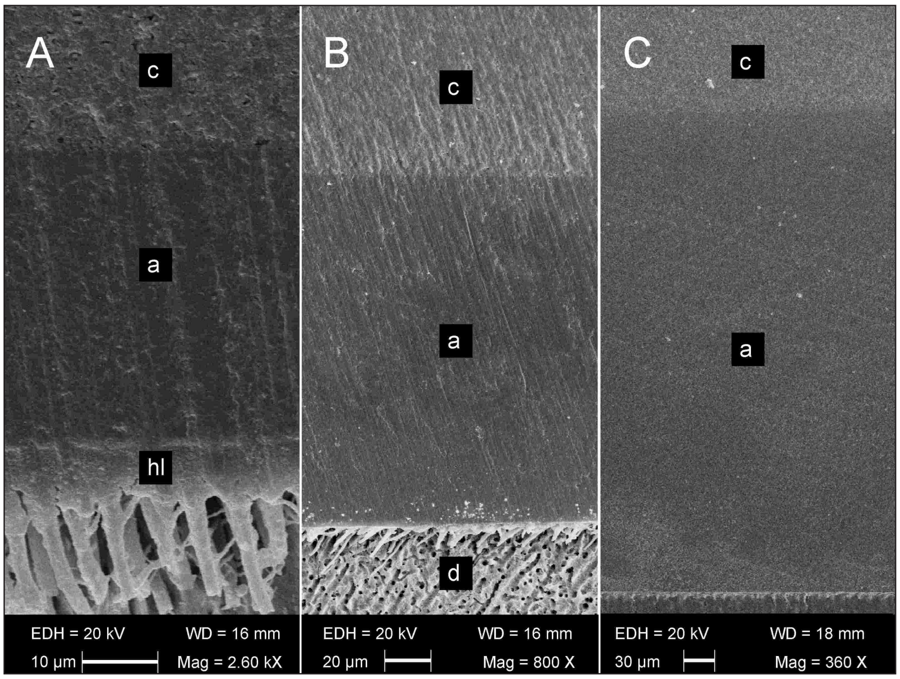

Figure 4: *Adhesive interface of the groups: (A) G1—conventional Single Bond application; (B) G2—2 applications of Single Bond and (C) G3—application of Scotchbond Multipurpose adhesive over Single Bond. c: composite; a: adhesive; d: dentin; hl: hybrid layer.*

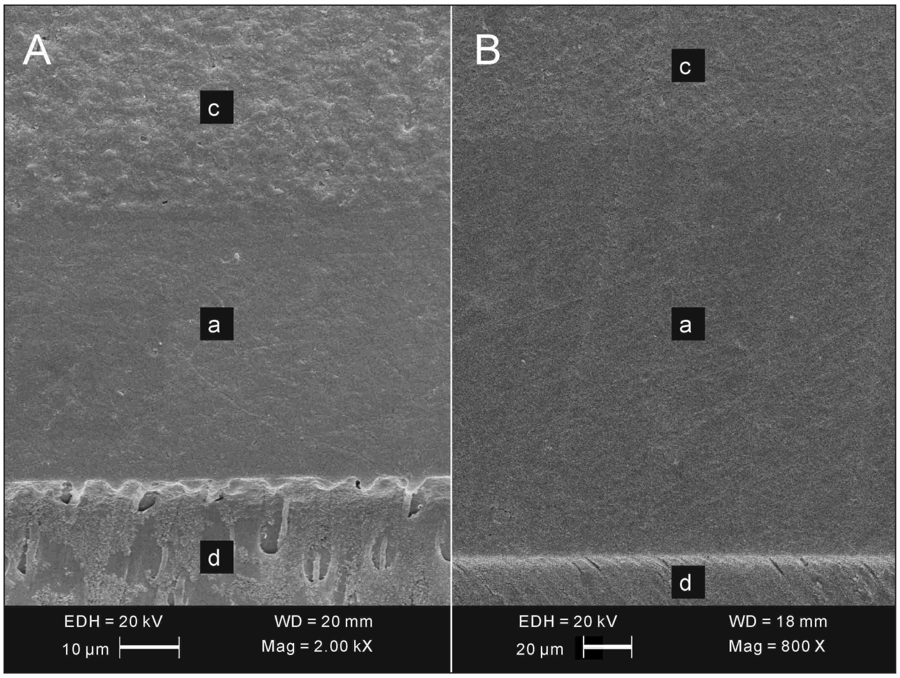

Figure 5: *Adhesive interface of the groups: (A) G4—conventional application of Clearfil SE Bond system and (B) G5—additional application of Clearfil SE Bond adhesive. c: composite; a: adhesive; d: dentin.*

Zheng and others,[13] using 2 consecutive layers of CE adhesive without photopolymerizing the first layer, found that higher bond strength values were obtained when the adhesive layer thickness was greater than

300 µm. This thickness was created with 2 additional layers and, as the thickness increased, bond strength values improved. These findings agree with those found in the current study, where the highest microtensile strength values were obtained with greater adhesive layers (SMP adhesive application). For the CE system, the application of more layers might improve the µTBS values, as the thickness might also be increased.

Rees and others[12] suggested that a thicker resin composite layer (above 80 µm) without filler particles would be necessary for a Class V restoration to resist stress created by polymerization shrinkage. In this study, the second layer application of all adhesive systems, except for the AP system, made it possible to obtain thicker adhesive layers. However, an additional application of the SB system, despite creating layers up to 80 µm thick, revealed a decrease in µTBS values. Both systems, AP and SB, have solvents in their composition. Therefore, according to the adhesive systems used in this study, the use of bonding agents that did not have solvents in their composition (SMP and CE) generated thicker adhesive layers without affecting µTBS values.

CONCLUSIONS

1. The µTBS values of Single Bond decreased when an additional adhesive layer was used.
2. When Scotchbond Multi-purpose adhesive was used over a polymerized adhesive layer of Single Bond, the µTBS increased.
3. The second application of a Clearfil SE Bond layer did not alter the µTBS.
4. The µTBS values for Adper Prompt were not improved by an additional adhesive application.

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