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Comparison of Alternate Protocols for Placing a Sixth Generation Dental Bonding Agent

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Abstract

Background: Dental bonding agent (DBA) manufacturers continue to make their products more attractive to practitioners by simplifying their product placement protocol. The purpose of this study was to compare shear bond strength (SBS) of alternate protocols of a sixth generation DBA

Methods: 140 caries free human molars were placed in 7 groups (n=20). Groups Part 1 A-C: manufacturers' recommended protocol and two alternate adhesive layer application techniques. Groups Part 2 A-D: manufacturers' recommended protocol and two alternate primer layer application techniques and manufacturer's total etch protocol. Deep dentin surfaces were exposed, polished, DBA's and universal nanohybrid composite placed. After 48hours, SBS was tested with universal testing machine. Samples of each group were prepared and imaged for descriptive assessment with CLSM.

Results: One-way ANOVA showed significant difference between groups In Part 1. (P<.001) Post hoc tests showed group A had significantly higher SBS than both adhesive alternative groups B and C. In Part 2.One-way ANOVA showed no significant difference (P=.067) between groups. Post hoc tests showed groups A-C with significantly higher SBS than group D but not among themselves.

Conclusions: The alternate primer placement protocols for the sixth generation DBA tested compared favorably with the manufacturers recommended protocol when comparing SBS.

Practical Implications: There may be some flexibility in placement of the sixth generation DBA Primer tested without significantly affecting its performance; however, the adhesive layer application should not be altered from manufacturer's recommendation.

Keywords: Dental adhesives; Self etch primer; Dentin bonding placement protocol

Introduction

Dental bonding adhesives (DBA's) have many different components/ingredients [1] as well as protocols on how to best use them for maximum performance. For many DBA's the primer placement and adhesive placement protocols differ. Studies have been done to compare manufacturer's protocol with alternate ones. Manuja et al. looked at the bonding efficacy of 1-step self-etch adhesives using enamel pre-etching and application of an additional hydrophobic resin layer. They found significant differences in some of their test groups [2]. Pashley et al. have done considerable studies using various protocols with dentin bonding involving dry bonding, water-wet bonding, and ethanol-wet bonding [3].

Ramesh et al. looked at the depth of resin penetration into enamel with three different types of enamel conditioning methods and used confocal laser scanning microscopy (CLSM) to assess the results. They found a significant difference in depth of resin penetration into enamel [4]. CLSM has been used in various studies to assess primer and adhesive penetration and thickness of various dental adhesives into dentin and enamel [5-8].

This study investigated the manufacturer's proposed protocol for Prelude Self Etch DBA and some placement protocol alternatives for the primer and adhesive. The purpose of this study was to compare alternate placement protocols of a sixth generation DBA to evaluate if the shear bond strength (SBS) to dentin would be affected.

Confocal laser scanning microscopy micrographs were additionally taken to illustrate representative pictures for each application protocol used in this study.

Methods

140 caries free extracted human third molars were collected prior to the study and stored in 0.1% Thymol at 4°C. Teeth were cleaned and observed for the absence of anomalies, caries, existing restorations, and deep crack lines and were randomly grouped into 7 groups (n=20). The study was conducted in two parts; Part 1 (groups1A-1C) used manufacturers' recommended protocol as control and two alternate adhesive layer application techniques, one having a thick adhesive layer and the other a thin adhesive layer. Part 2 (Groups 2A-2D) used manufacturers' recommended protocol as control and two alternate primer layer application techniques, one with additional primer application, one with etch and rinse plus self etch primer, and the manufacturer's total etch protocol technique without primer. The Valo LED light curing unit (Ultradent Products Inc, South-Jordan, UT, USA) was used throughout the study using a continuous curing protocol. The same air syringe unit was used to apply air pressure to reduce the thickness of the adhesive layer-the measured pressure was in the range of 35psi.

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Part 1: Adhesive protocol descriptions

Group 1A: Control (Manufacturer's recommended protocol)

1. Apply Primer to damp dentin scrub 10s
2. 10s dry with air to gently evaporate solvent (5s light then 5s brisk until no visible fluid movement) surface need not remain shiny
3. Apply Adhesive , scrub for 10s
4. Evaporate solvent with gentle air stream 5s, increase air pressure to thin adhesive 5s
5. Light cure 10s
6. Herculite Ultra placed in Watanabe bonding jig in two increments and light cured 40s each

Group 1B: Thin adhesive layer (same protocol as in group A with the exception of step 4)

1. Apply Primer to damp dentin scrub 10s
2. 10s dry with air to gently evaporate solvent (5s light then 5s brisk until no visible fluid movement) surface need not remain shiny
3. Apply Adhesive , scrub for 5s apply additional layer scrub 5 s
4. Evaporate solvent with gentle air stream for 5 s, increase air pressure to thin adhesive 5-8s until no visible fluid movement (like primer)
5. Light cure 10s
6. Herculite Ultra placed in Watanabe bonding jig in two increments and light cured 40s each

Group 1C: Thick adhesive layer (protocol as in group A with the exception of step 4)

1. Apply Primer to damp dentin scrub 10s
2. Dry with air to gently evaporate solvent (5s light then 5s brisk until no visible fluid movement) surface need not remain shiny
3. Apply a thick layer of adhesive, scrub for 10s
4. Evaporate solvent with gentle air stream for 5 s, increase air pressure slightly to thin adhesive 5s but leave a moveable layer)
5. Light cure 10s
6. Herculite Ultra placed in Watanabe bonding jig in two increments and light cured 40s each

Part 2: Primer protocol descriptions

Group 2A: Control (Manufacturer's recommended protocol)

1. Apply Primer to damp dentin scrub 10s
2. 10s dry with air to gently evaporate solvent (5s light then 5s brisk until no visible fluid movement) surface need not remain shiny
3. Apply Adhesive , scrub for 10s (manufacturers recommended protocol)
4. Evaporate solvent with gentle air stream 5s, increase air pressure to thin adhesive 5s
5. Light cure 10s
6. Herculite Ultra placed in Watanabe bonding jig in two increments and light cured 40s each

Group 2B: Additional primer application (protocol as in Group A. with exception of step 1)

1. Apply Primer to damp dentin scrub 20s (10s scrub re-dip applicator 2nd 10s scrub)
2. Dry with air to gently evaporate solvent (surface need not remain shiny)
3. Apply Adhesive , scrub for 10s
4. Evaporate solvent with gentle air stream , increase air pressure to thin adhesive
5. Light cure 10s
6. Herculite Ultra placed in Watanabe bonding jig in two increments and light cured 40s

Group 2C: Additional dentin etches (same protocol as Group A. with exception of step 1)

1. 10s etch and rinse with 37% PA, Air dry to leave dentin slightly moist
2. Apply Primer to damp dentin scrub 10s
3. Dry with air to gently evaporate solvent (surface need not remain shiny)
4. Apply Adhesive , scrub for 10s
5. Evaporate solvent with gentle air stream , increase air pressure to thin adhesive
6. Light cure 10s
7. Herculite Ultra placed in Watanabe bonding jig in two increments and light cured 40s each

Group 2D: Manufacturer's *Total Etch* protocol

1. etch 10s with 37% PA and rinse, Air dry to leave dentin moist
2. apply Adhesive , scrub for 10s
3. evaporate solvent with gentle air stream, increase air pressure to thin adhesive
4. light cure 10s
5. Herculite Ultra placed in Watanabe bonding jig in two increments and light cured 40s

The occlusal surfaces were removed from all specimens on a diamond disc model trimmer (Ray Foster Dental Supplies, Lynwood, CA, USA) exposing deep dentin surface, which was polished sequentially with 240 and 600 grit SiC paper. After placement of DBA's all groups had a universal nanohybrid dental composite (Herculite Ultra A2, Kerr, Orange, CA, USA) placed using the Watanabe jig (Spec. ISO-TS-11405), and then stored in 100% humidity at 35°C for 48 hours. The bond area of the jig was 3mm in diameter and established by an aperture of self-sticking Mylar material, 0.002" that had been pre-cut with lasers. Next the SBS was tested using "Watanabe" single plane lap shear test with a universal testing machine (MTS Systems Inc., Eden Prairie, MN, USA).

Three additional samples of each group were prepared with a 3mm deep, 3mm wide preparation going horizontally from mesial to distal. These were used for the descriptive assessment with CLSM.

The CLSM specimens had the primer labeled with Rhodamine B (0.16 mg/ml)(Sigma-Aldrich, St. Louis, MO, USA) and the adhesive labeled with Fluorescein (0.16 mg/ml) (Sigma-Aldrich, St. Louis, MO, USA). The CLSM specimens were prepared by placing a 1.0 mm layer of flowable resin (Accolade, Danville, San Ramon, CA, USA) over the bonding adhesive and light cured for 40 seconds. They were then embedded in self cure acrylic and sectioned horizontally into 1 mm slabs using a slow-speed water-cooled diamond saw. The resin–dentin slabs were then polished using 600-grit SiC paper. Each resin-dentin interface was investigated and images representing the most common features of primer/adhesive penetration and thickness captured and recorded. The imaging procedures were performed using a confocal laser scanning microscope (Zeiss 710 CLSM,) equipped with a 63×/1.4 NA oil immersion lens using 488 nm argon/helium and a 633 nm krypton ion laser illumination. The z-axis scan of the interface surface was converted into pseudo-color for better visualization, and compiled into both single and topographic projections using Zeiss CLSM image-processing software.

Statistical analysis was performed with IBM SPSS version 20.0 computer soft ware using one-way analysis of variance (ANOVA) for the comparison of SBS among the experimental groups. The statistical significance level was set as α=0.05.

Results

One way ANOVA showed significant difference between the groups in Part 1. ($p<.001$, 95% CI=23.97-28.20). Post hoc tests showed that group 1A had significantly higher SBS than both the adhesive alternative groups 1B and 1C (Figure 1 and 2). Note the variance as shown in Table 1 was least for the manufacturers' protocol (group 1A).

In Part 2 One-way ANOVA showed no significant difference ($p=.067$; 95%CI=25.45-27.93) between the groups. But post hoc tests showed that groups 2A, 2B, and 2C had significantly higher SBS than group 2D but not among themselves (A: P=0.042 CI=-6.96- -0.13; B: P=0.023 CI= -7.39- -0.56; C: P=0.026 CI=-7.29- -0.47). Group D protocol was the manufacturer's total etch two step using adhesive only (no primer). Note that the variance as shown in Table 2 was least for the additional primer application protocol (group 2B) and greatest for the manufacturer's total etch two-step protocol (group 2D).

Figures 3-7 illustrate the representative CLSM microradiographs for each protocol used in this study. The penetration of the primer into the dentinal tubules (red) and the varying thickness of the adhesive layer

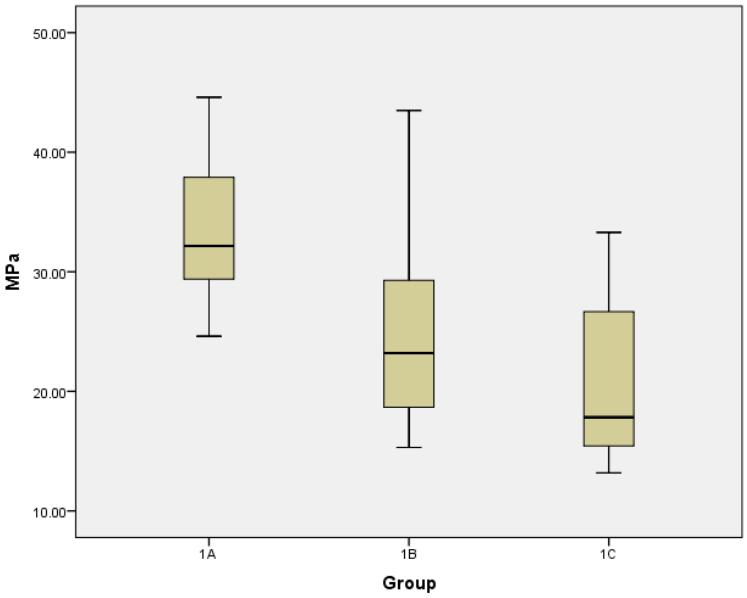

Figure 1: Boxplots of Shear Bond Strengths by Group: Part 1. Adhesive Application Protocols.

Group	N	Mean SBS (MPa)	Standard Deviation	Standard Error	95% Confidence interval
1A manufacturer protocol	20	33.24	5.67	1.27	30.59-35.89
1B thin adhesive layer	20	24.07	7.04	1.57	20.78-27.36
1C thick adhesive layer	20	20.95	6.41	1.43	17.95-23.94

Table 1: Descriptive Statistics Part 1.

Group	N	Mean SBS (MPa)	Standard Deviation	Standard Error	95% confidence interval
2A manufacturer protocol	20	27.40	5.33	1.20	24.89-29.88
2B additional primer application	20	27.82	3.91	.88	25.98-29.65
2C additional dentin etch	20	27.72	4.60	1.03	25.57-29.87
2D manufacturer's total etch protocol	20	23.84	7.24	1.62	20.45-27.23

Table 2: Descriptive Statistics Part 2.

according to the application protocol are easily visible. Noteworthy is the facilitated penetration of the primer with additional dentin etching in group 2C (Figure 6).

Discussion

With the evolvement of newer generations, DBA's primer and adhesive placement protocols differ widely. This can make the bonding process quite confusing to the practitioner especially if one does not closely follow the research and or the manufacturers' recommendations and tries the "one size fits all" approach for DBA usage. Part of the rationale for this study was to evaluate the flexibility of the DBA used in this study. In other words; how forgiving is the material, when manufacturer's instructions are not exactly followed or modified.

Part 1 of this study dealt with variant protocols of adhesive application with the primer being placed according to manufacturer's recommended protocol in all groups. Part 2 of this study consisted of the adhesive being placed according to manufacturer's recommended protocol in all groups and the primer application using variant protocols.

The results for part 1 showed that the manufacturer's recommended protocol (group 1A) had a significantly higher bond strength than both the thin adhesive layer (group 1B) and the thick adhesive layer (group

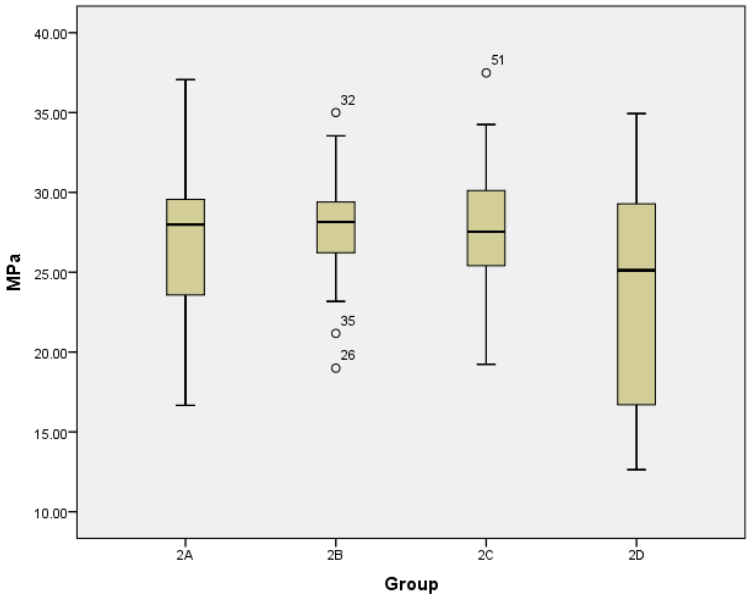

Figure 2: Boxplots of Shear Bond Strengths by Group: Part 2. Primer Application Protocols.

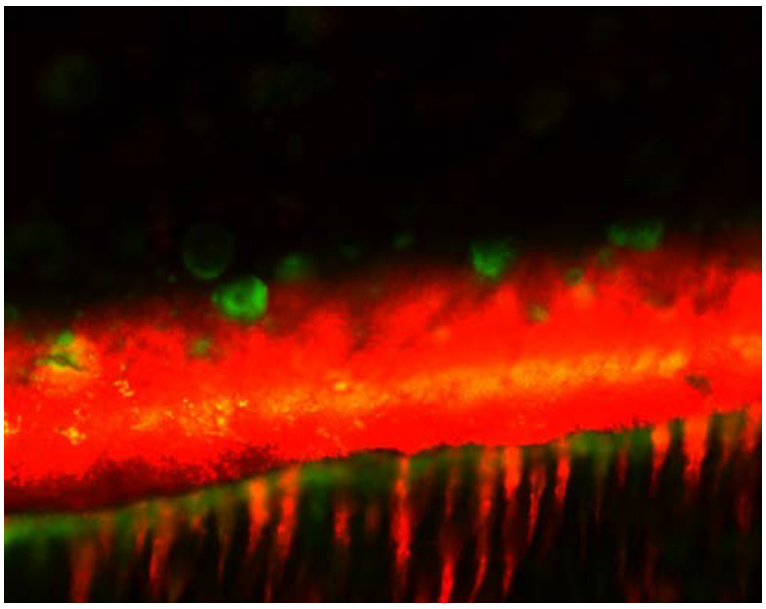

Figure 3: CLSM micrograph of manufacturer's recommended standard protocol.

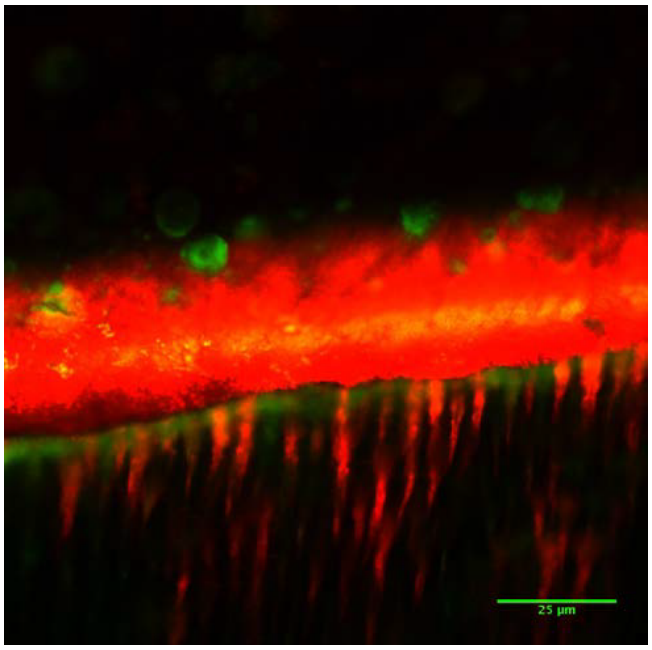

Figure 4: CLSM micrograph of thin adhesive layer protocol.

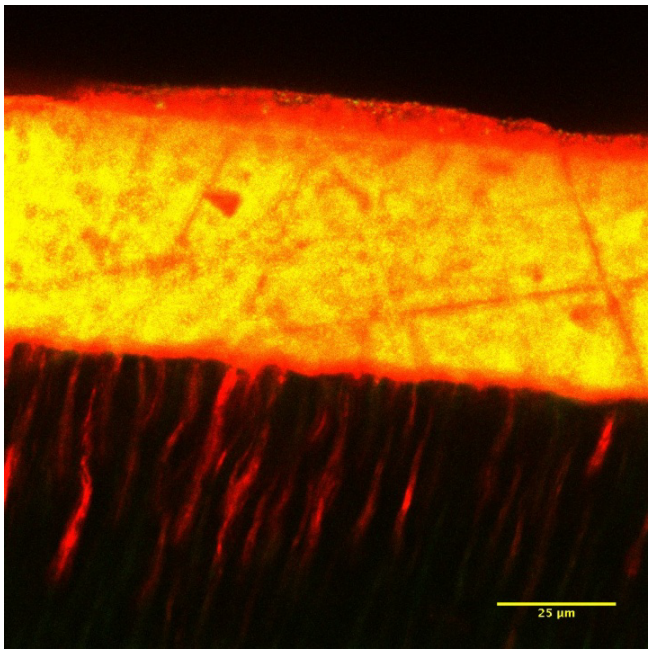

Figure 5: CLSM micrograph of thick adhesive protocol.

1C), however the thin layer of adhesive averaged a bond strength approximately 13% higher than the thick layer of adhesive. A study done by Ma et al. showed that multiple coatings could improve the bond durability of one-step self-etching adhesive to primary dentin when applying a thick adhesive layer by placing 3 layers of adhesive before light-curing [9]. Lodovici et al. did not find the same in their study. They found that application of an additional hydrophobic resin layer "acting as an intermediate flexible layer, did not minimize the damage caused by thermal/mechanical load cycling"; however, their study was done using a three-step etch & rinse and two-step self-etch system while most of the current literature regarding the application of an additional hydrophobic resin layer has demonstrated the effect to be beneficial for simplified one-step self-etch adhesives bonded to dentin [10]. Albuquerque et al. had similar results in their study [11]. They observed that the additional application of adhesive had a greater effect on dentin than enamel and a greater effect with one bottle adhesives than the two-step self-etch adhesive used as the control. The results of this study are similar with the Albuquerque study in that the extra application of adhesive weakened the bond of the two-step self-etch

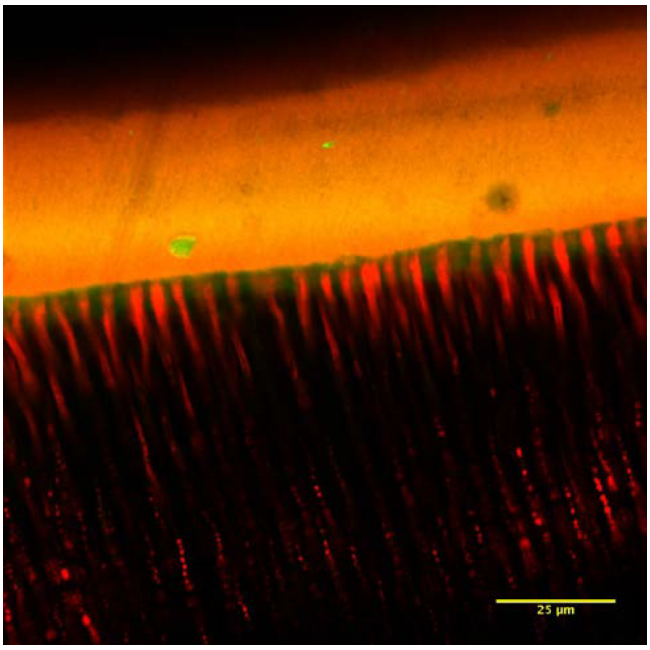

Figure 6: CLSM micrograph of etching followed by manufacturer's recommended standard protocol.

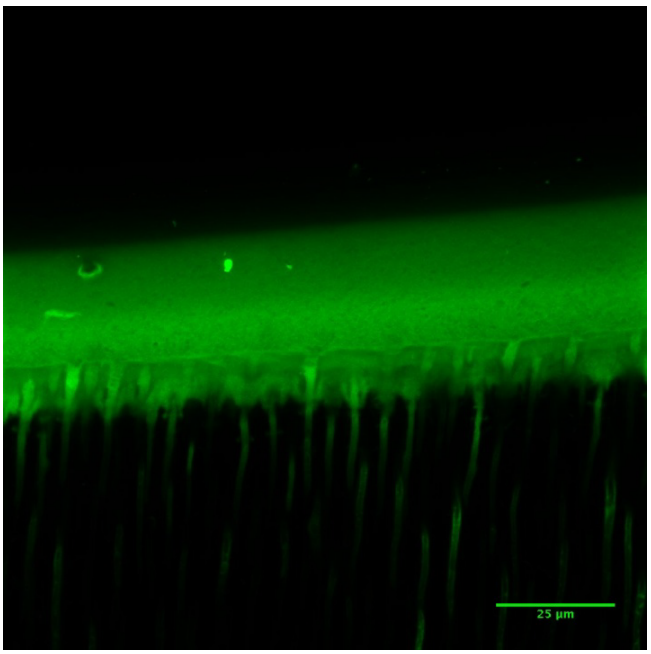

Figure 7: CLSM micrograph of manufacturer's recommended total etch protocol.

adhesive, as was the case with the two-step self-etch adhesive DBA used in this study.

As for part 2 of this study, the bond strength was almost identical with groups2A, 2B and 2C and with the non-primer group 2D (manufacturer's etch and rinse 2 step protocol) having a significantly weaker bond. Although groups' 2A-2C bond strengths were very similar, group 2B, with additional primer application had slightly higher bond strength but significantly less variability. In the studies mentioned above the additional adhesive application with one-bottle adhesive is thought to help form a thicker and denser hybrid layer between the DBA and dentin. The representative CLSM taken in our study following a labeling protocol by De Oliveria et al. [7] showed a thicker adhesive layer thickness but could not detect any differences in hybrid layer thickness or quality. However, the incorporation of the fluorescent probes into the primer or adhesive resin is almost entirely a physical phenomenon and not a molecular event so that there is a possibility that the fluorescent probe might diffuse out of the material, making interpretation of morphological studies difficult [12].

The clinical implication of this study looking at varying adhesive versus primer protocols in a two step self-etch adhesive DBA suggested that it is advised to follow manufacturer's direction and that following the adhesive protocol was more critical than the primer protocol.

Conclusion

Within the limitations of this study it can be concluded that Prelude 6th generation DBA, when placed as described by the manufacturer protocol resulted in very good bond strength. In addition, we observed that variations in adhesive placement affected bond strengths more than did variations in dentin primer placement. Finally, it seems that this is a user-friendly DBA system and the primer placement protocol is somewhat flexible in achieving acceptable bond strengths.

Acknowledgment

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Clinical Relevance

The success of a resin composite restoration depends largely on the integrity of the placement of the dental bonding agent (DBA). This study looks at variant placement protocols and their affect on the shear bond strength of a sixth generation DBA.

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