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Effect of Multiple Coatings of One-step Self-etching Adhesive on Microtensile Bond Strength to Primary Dentin

Lin Ma¹, Jian-feng Zhou², Jian-guo Tan², Quan Jing¹,
Ji-zhi Zhao¹, and Kuo Wan^{1*}

¹Department of Stomatology, Peking Union Medical College Hospital, Chinese Academy of
Medical Sciences & Peking Union Medical College, Beijing 100730, China

²Department of Prosthodontics, Peking University School and Hospital of
Stomatology, Beijing 100081, China

Key words: self-etching adhesive; microtensile bond test; primary dentin; multiple coatings

Objective To investigate the effect of multiple coatings of the one-step self-etching adhesive on immediate microtensile bond strength to primary dentin.

Methods Twelve caries-free human primary molars were randomly divided into 2 groups with 6 teeth each. In group 1, each tooth was hemisected into two halves. One half was assigned to control subgroup 1, which was bonded with a single-step self-etching adhesive according to the manufacturer's instructions; the other half was assigned to experimental subgroup 1 in which the adhesive was applied three times before light curing. In group 2, the teeth were also hemisected into two halves. One half was assigned to control subgroup 2, which was bonded with the single-step self-etching adhesive according to the manufacturer's instructions; the other half was assigned to experimental subgroup 2 in which three layers of adhesive were applied with light curing each successive layer. Microtensile bond strength was immediately tested after specimen preparation.

Results When the adhesive was applied three times before light curing, the bond strength of the experimental subgroup 1 ($n=33$, 57.49 ± 11.61 MPa) was higher than that of the control subgroup 1 ($n=31$, 49.71 ± 11.43 MPa, $P < 0.05$). When using the technique of applying multiple layers of adhesive with light curing each successive layer, no difference of immediate bond strength was observed between the control subgroup 2 and the experimental subgroup 2 ($P > 0.05$).

Conclusion Multiple coatings of one-step self-etching adhesive can increase the immediate bond strength to primary dentin when using the technique of light-curing after applying three layers of adhesive.

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THE self-etching system is attractive in pediatric dentistry because it requires 'fewer steps' and 'less operation time' compared with the total-etching system. Recently, the advent of one-step self-etching adhesives has further improved the efficiency in clinical procedures by simplifying procedural steps and reducing the complexity of the technique.^{1, 2} Although one-step self-etching adhesives have simplified application procedures and lowered technique sensitivity, some previous studies have identified some concerns about the performance of these adhesives such as reduced dentin bond strength.³⁻⁶

The lack of a relatively hydrophobic bonding agent as a separate final step in the adhesive procedure is one of the main reasons for the poor performance of one-step self-etching adhesives. Because of the relatively high percentage of hydrophilic monomers blended within their formulation, the adhesive interface acts as a semipermeable membrane after polymerization. Fluids can cross the interface as a result of osmotic pressure gradients.⁷ Therefore, one-step self-etching adhesives have increased water sorption, which promotes polymer swelling and other water-mediated degradation phenomena.^{8, 9}

Several prior studies have pointed out that multiple coatings of one-step self-etching adhesives can increase resin infiltration into the collagen network as well as reduce water transudation from underlying dentin, thus increase dentin bond strength.¹⁰⁻¹³ Although there have been studies on the effects of multiple coatings on bond strength of resin to permanent dentin, so far as we know, there is no study to evaluate the effects of multiple coatings of a one-step self-etching adhesive system on the bond strength to primary dentin.

It has been pointed out that the density and diameter of dentinal tubules is higher in primary dentin than that in permanent dentin. The amount of surface moisture was also found to be higher in primary dentin. All these factors alter the effectiveness of the dentin conditioners, hence affecting the bond strength.^{14, 15} However, people often transfer the knowledge about permanent teeth to primary teeth, regardless of these substantial microstructural differences between permanent and primary dentin. Therefore, some of the studies on permanent teeth, such as the technique of multiple coatings which might alter the conditions of bond surface and the quality of the hybrid layers, are worthy to be ascertained on primary teeth. The aim of this study was to assess the effect of multiple coatings of a one-step self-etching adhesive on immediate microtensile bond strength (μ TBS) to primary dentin. The null hypothesis in our study tested was that multiple coatings of

one-step self-etching adhesive have no effect on immediate μ TBS to primary dentin.

MATERIALS AND METHODS

Tooth preparation

Twelve caries-free second primary molars, which had been extracted because of prolonged retention, were collected with informed consent signed by the children's parents. The study protocol was reviewed and approved by the Ethics Committee for Human Studies, Peking University, Beijing, China. The teeth were stored in 0.9% NaCl containing 0.02% sodium azide at 4°C for no more than one month.

The occlusal enamel of each tooth was removed. The teeth were hand polished on a wet 600-grit silicon carbide paper for 30 seconds to create a realistic smear layer on the surface of the occlusal mid-coronal dentin.

Experimental design

A single-step self-etching adhesive—Clearfil S3 Bond (Kuraray Medical, Osaka, Japan, LOT NO. 00143A) was used in this study. The composition of Clearfil S3 Bond is shown as follows: water, 10-methacryloyloxydecyl dihydrogen phosphate (MDP), bisphenol A diglycidyl methacrylate (Bis-GMA), 2-hydroxyethyl methacrylate (HEMA), hydrophobic dimethacrylate, camphorquinone, ethyl alcohol, and silanated colloidal silica.

The 12 teeth were randomly divided into 2 groups, 6 teeth in each group.

Bonding procedures

Group 1: Self-controlled method was performed in each tooth: each of the 6 teeth was hemisected into 2 halves using a low-speed water-cooled saw equipped with a diamond-impregnated disk (Isomet 1000; Buehler Ltd., Lake Bluff, IL, USA). Each half was randomly assigned to the control (C1) or experimental (E1) subgroup respectively. The C1 subgroup was bonded with the single-step self-etching adhesive according to the manufacturer's instructions: a generous amount of adhesive was applied to the dentin surface using a sponge ball. After leaving the adhesive undisturbed for 20 seconds, the adhesive was uniformly spread with a strong air stream for 10 seconds and then light-cured for 10 seconds using a halogen light-curing unit with an output of 700 mW/cm². For the E1 subgroup, adhesive was applied to the dentin surface, leaving undisturbed for 20 seconds, spreading with a strong air stream for 10 seconds. These steps were done repeatedly for 3 times, then the adhesive was light-cured

for 10 seconds. After an 8-mm-height composite resin block (Clearfil AP-X; Kuraray, Osaka, Japan) was built up on the bonded surfaces by 5 or 6 sequential increments of resin, each one was light-cured for 20 seconds.

Group 2: The other 6 teeth were also hemisected into 2 halves. The control half teeth (C2 subgroup) were bonded with the single-step self-etching adhesive according to the manufacturer's instructions. For the experimental half teeth (E2 subgroup), adhesive was applied to the dentin surface, leaving undisturbed for 20 seconds, spreading with a strong air stream for 10 seconds, then light-cured for 10 seconds. This procedure was repeated for 3 times, followed by creation of composite buildups.

Microtensile bond testing

After stored in distilled water at 37°C for a week, the bonded teeth were longitudinally cut across the bonded interface in sections perpendicular to the bonded surfaces with a diamond saw (Isomet 1000), to produce a series of 0.8 mm×1.5 mm×8.0 mm beams. Four to six beams were obtained from each preparation. These beams were then trimmed into hourglass-shaped specimens with a cross-sectional area of approximately 0.6 mm². These specimens were stored in distilled water and tested after 24 hours. Each specimen was individually fixed to a custom-made testing jig with a cyanoacrylate glue (Universal Instant Adhesive; Henkel Adhesives Co. Ltd., Shantou, China). The specimen was then subjected to tensile load at a crosshead speed of 1.0 mm/min until failure (Autograph DCS-5000, Shimadzu, Japan). The data were recorded in Newtons (N) and later converted to megapascals (MPa=N/mm²) (divided by cross-sectional area).

Debond pathway determination

Both surfaces of each fractured specimen were observed under a stereomicroscope (Olympus 220670; Tokyo, Japan) with 40× magnification to record the failure modes. The fracture modes were classified as follows: (1) cohesive failures in the composite resin were defined as the fracture occurred exclusively within the resin composite; (2) failures in the adhesive joint were confirmed if the fracture site

was entirely within the adhesive; (3) cohesive failures in dentin were the fracture occurring exclusively within the dentin; and (4) mixed failures were verified if the fracture site continued from the adhesive into either the resin composite or dentin.

Statistical analysis

We tested all data using SPSS statistical software (version 16.0, Chicago, USA). The immediate μ TBS was expressed as mean±SD. Two-way analysis of variance (ANOVA) and post hoc Tukey tests were used to compare the effects of the dentin treatments (control group vs. experimental group) on bond strength. Statistical significance was preset at $\alpha=0.05$. The statistical unit was beams, not teeth.

RESULTS

Of a total of 129 specimens, only 3 specimens failed during the pretest phase. All these 3 specimens failed during being trimmed into hourglass-shaped specimens. One was from C1 subgroup, one from E1 subgroup, and one from C2 subgroup. The missing data were deleted. Finally, the sample size of C1, E1, C2 and E2 subgroups was 31, 33, 32, and 30, respectively.

μ TBS

In group 1, the immediate μ TBS of the E1 subgroup was significantly higher than that of the C1 subgroup (57.49±11.61 vs. 49.71±11.43 MPa, $P<0.05$). In group 2, there was no significant difference between the C2 and E2 subgroups (50.30±10.80 vs. 48.85±9.44 MPa, $P>0.05$). The immediate μ TBS of the E1 subgroup was significantly higher than that of the C2 as well as E2 subgroups (both $P<0.05$).

Distribution of the fracture mode

Table 1 summarizes the distribution of the fracture modes. Adhesive joint failures were the most common fracture pattern observed in both the control and experimental subgroups.

Table 1. Distribution of the fracture mode of the four study groups

Group	Sample size	Fracture mode [<i>n</i> (%)]			
		Cohesive failures in the composite resin	Failures in the adhesive joint	Cohesive failures in dentin	Mixed failures
Group 1	C1 subgroup	3 (9.68)	24(77.42)	1(3.23)	3(9.68)
	E1 subgroup	3 (9.09)	26(78.79)	2(6.06)	2(6.06)
Group 2	C2 subgroup	4(12.50)	25(78.13)	0(0)	3(9.38)
	E2 subgroup	1(3.33)	27(90.00)	0(0)	2(6.67)

DISCUSSION

As shown in this study, the immediate bond strength of the E1 subgroup was higher than C1 subgroup, indicating that applying 3 layers of adhesive before light curing can increase the immediate bond strength of resin to primary dentin. However, no difference was observed between the C2 subgroup and E2 subgroup, indicating that the technique of applying 3 layers of adhesive with curing each successive layer can not improve the immediate bond strength.

In most previous studies, in which multiple coating technique was used on permanent teeth, applying multiple layers of adhesive before light curing can increase the immediate bond strength.^{12,16-19} Similar results were obtained in this study, although different adhesive and different adhesive layers were used. In the case of one-step self-etching adhesives, penetration of the adhesive into dentin simultaneously occurs with demineralization of the dentin. When the resin fails to completely infiltrate deeper portions of the demineralized dentin, the bond between resin and dentin might be weakened.²⁰⁻²² Applying multiple consecutive coatings prolongs adhesive application time, which might allow resin monomer to penetrate into the total depth of the demineralized dentin.^{12,16} Besides, Clearfil S3 Bond contains a significant amount of water and solvent which are expected to be removed by air blowing after application of the adhesive.¹⁰ Multiple coatings also prolong air-drying time, therefore can remove residual water and solvent more effectively.¹³ This has been confirmed by Erhardt *et al*¹³ and Hashimoto *et al*²³ that the prolonged application and subsequent solvent evaporation may improve resin infiltration within the exposed collagen fibers. Therefore, the use of multiple adhesive applications before curing, as was done in this study, allows more time for adhesive diffusion and removal of residual water, thereby, improving resin infiltration and crosslinking of the adhesive comonomers within the hybrid layer.

It has been well accepted that there are significant microstructural differences between primary dentin and permanent dentin.²⁴ As primary dentin has greater tubule density and larger tubule diameters, acid etching would give larger tubule lumens, thus decreasing the solid dentin available for bonding.¹⁴ Besides, primary dentin has higher surface wetness due to its closer vicinity to the pulp chamber with the presence of higher amounts of water.²⁵ Except for improving resin infiltration and removing residual solvent effectively, applying multiple layers of adhesive before light curing can reduce water transudation from

underlying dentin.^{10,11,13} Therefore, it might be more valuable to apply multiple coatings on primary dentin than on permanent dentin as primary dentin has more microcanals and higher surface wetness.

When each successive layer is light cured, the adhesive layer becomes thicker without changing the quality of the hybrid layer, which can create a relatively thick intermediate layer with low elastic modulus between dentin and composite. It has been speculated that as the component overlying the hybrid layer, the adhesive layer might help to preserve the integrity of the hybridized dentin, protect it from polymerization shrinkage stresses, act as a stress absorbing layer, and contribute to improving and maintaining bond strength.²⁶⁻²⁸ However, each adhesive system has an ideal thickness to reduce interface stress while preserving the interface integrity.¹¹ And quite different results were achieved in different studies. D'Arcangelo *et al*¹¹ reported an excess of adhesive layer thickness could negatively influence the strength. Pashley *et al*²⁹ and de Silva *et al*³⁰ demonstrated an increased bond strength using the technique of applying multiple layers of adhesive then curing each successive layer. This is probably due to the differences in procedures, application modalities, and compositions (solvent agents and filling materials) of the various commercially available bonding systems used.³¹ It is worth emphasizing that, to date, no study has successfully established a positive correlation between the thickness of the resin-infiltrated layer and bond strength, suggesting that the quality, rather than the thickness of the resin-infiltrated layer, is more important for bond strength measurements.^{23, 32}

μ TBS testing is considered the most credible method of evaluating dentin adhesion. With using small sized specimens (1 mm²), it offers several advantages including optimal stress distribution at the resin/dentin interface, more adhesive failures (that is, true bond strength values), fewer cohesive failures, and a higher interface bond strength.³³ On the other hand, it has been reported that cohesive fractures are not unusual in primary dentin, which can be explained because the micro-hardness of deep dentin is low.³⁴ In our present study, failure patterns are mainly adhesive joint failures in both the control and experimental subgroups. This was possibly because of the smaller cross-sectional area obtained in this study, which on average was approximately 0.6 mm². Besides, the beams were trimmed into hourglass-shaped specimens, which had the minimum cross-sectional area at the resin/dentin interface.

On the whole, the increased bond strength using multiple coatings of adhesive before light curing in the present

study might be due to increased resin infiltration into the demineralized dentin and increased removal of residual water rather than the increased thickness of the overlying adhesive layer.¹⁰ These results also illustrate how important bonding technique is to produce optimal resin-dentin bonds. Simple changes in bonding technique, such as applying more layers of all-in-one adhesives, can lead to significant increases in initial bond strength.

However, this *in vitro* study needs further *in vivo* implementation, because this laboratory test was done using extracted teeth without regarding the pulpal pressure and presence of dentinal fluid under realistic physiological conditions, which may adversely affect dentin bonding.^{35, 36} In addition, whether multiple coatings of adhesive can improve the long-term durability of resin-dentin bonds remains to be determined. Thus, long-term clinical studies are necessary to evaluate the efficacy and durability of these self-etching bonding systems.

In conclusion, within the limitations of this study, we conclude that applying 3 layers of one-step self-etching adhesive (Clearfil S3 Bond) before light curing can increase the μ TBS of resin to primary dentin.

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