

Effects of Single and Double Application of Immediate Dentin Sealing Techniques on Marginal Leakage and Microtensile Bond Strength of Resin Cement

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Abstract

To evaluate the effects of single and double application of immediate dentin sealing (IDS) techniques on marginal leakage and microtensile bond strength of resin cement.

Twenty-five extracted third molars were divided into five experimental groups in order to test the marginal leakage and microtensile bond strength (μ TBS) of direct bonding, single and double application of IDS techniques using three-step etch-and-rinse (1TE&2TE), and universal (1U&2U) adhesive systems. For the single application of the IDS technique, dental adhesive was applied on the prepared dentin prior to temporary cementation. For the double application, dental adhesive was re-applied after polymerization of the first layer and before temporary cementation. After removing the temporary cementation, the dentin surface was cleaned with an excavator and pumice slurry. Resin cement was used to bond a composite rod to the dentin surface. All specimens were cyclic loaded (50N, 2Hz, 50,000 cycles) and immersed in marginal leakage solutions, and then sectioned into two parts. The first half was cut into small square beams for μ TBS testing, while the other half was examined under a light microscope for marginal leakage.

The 1TE and 1U groups provided significantly higher μ TBS (23.56 \pm 3.26 MPa, 21.51 \pm 1.30 MPa) than the direct bonding technique (13.56 \pm 2.74 MPa) ($p < 0.001$). There was significant reduction of μ TBS (2TE = 21.41 \pm 1.59 MPa, 2U = 17.51 \pm 2.80 MPa) ($p < 0.01$) after applying the second layer of adhesive. The marginal leakage was not significantly different in all groups.

The two-layer IDS technique using either three-step etch-and-rinse or self-etch universal adhesive did not show any advantage in the μ TBS testing or marginal leakage over the one-layer IDS techniques, which showed significantly greater μ TBS than the direct bonding technique.

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Introduction

The use of immediate dentin sealing (IDS) technique has been claimed to be able to prevent a collapse of collagen network and form a complete hybrid layer, which effectively transfers load to dentin underneath. In contrast, direct bonding technique does not provide a stress-dissipating layer, and more deterioration of resin-dentin interface occurs after mechanical loading¹.

Before provisional cementation, the IDS technique was proposed for sealing freshly prepared dentin with dental adhesive²⁻⁵ to

improve the bond strength of resin cement. This technique not only provides a complete hybrid layer, which potentially better withstands long-term exposure to thermal and functional loads², but also reduces the contamination of temporary cementation on dentin surface, which might compromise the bond strength of resin cement and fixed restorations. The suggested IDS technique's bonding protocol is to apply a single layer of adhesive to dentin surface⁵. To overcome the limitations of dental adhesives, other bonding procedures that improve the quality of resin-dentin bond have been suggested⁶. The use of multiple additional layers of adhesive was one of these alternative bonding techniques^{6,7}.

The literature⁷⁻¹¹ revealed that multiple coats of dental adhesive systems could improve the bond strength of restorations and reduce

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nanoleakage. Hashimoto et al.⁸ reported that up to four consecutive coatings of total-etch adhesives without light curing of each coat increased bond strength, while Ito et al.⁷ revealed that bond strength increased with the number of coatings up to three layers, especially if each layer was light cured. However, there was a concern that excessive thickness could adversely affect microtensile bond strength^{11,12}. The publications that have compared multiple coats of adhesive layers as an immediate dentin sealing technique before permanent cementation with resin cement are still limited. Therefore, this study aimed to evaluate the effects of one-layer and two-layer IDS techniques on marginal leakage and microtensile bond strength.

Materials and methods

The Human Experimentation Committee of the Faculty of Dentistry, Chiang Mai University, Thailand approved the use of human tissue in this study No. 45/2021. Twenty-five caries-free human third molars were collected after fresh extraction under patient consent. The remaining soft tissue on the surface of tooth specimen was mechanically removed. The teeth were immersed in 1% chloramine T trihydrate solution for one week and stored in distilled water at 37 °C until used.

The whole tooth sample was embedded in epoxy resin with a buccal surface upward. A flat dentin surface, 5 mm in diameter, parallel to its long axis was prepared on the buccal surface using a low-speed cutting machine with water cooling (Isomet 1000® precision low-speed saw, Buehler, IL, USA). The cut surface was polished with 400-grit silicon carbide paper under water coolant.

Twenty-five tooth specimens were divided equally into five groups: one for the control group in which the composite was bonded directly to the dentin surface without dental adhesive coating (group D), two experimental groups: one-layer IDS applied using a three-step etch-and-rinse adhesive system (OptiBond FL, Kerr Corporation, Orange, CA, USA) (group 1TE) and a universal adhesive system (Single Bond Universal Adhesive, 3M ESPE, Seefeld, Germany) (group 1U), and in the other two groups, the second layer IDS was applied using the same bonding systems as the one-layer IDS (groups 2TE and 2U).

In the 1TE group, the prepared tooth surface was treated with 37% phosphoric acid for 15 seconds, rinsed with running water for 15 seconds and air-dried. The primer was applied on the etched surface with a micro-brush for 15 seconds followed by gentle air blowing. The bonding agent was painted onto the primed surface, followed by gentle air blowing and activated with high intensity light for 20 seconds. For the 2TE group, the second layer of bonding agent was applied followed by light activation. In the same way, in the 1U group, self-etched primer bonding agent was applied on prepared dentin for 20 seconds, followed by air-drying and light activation for 20 seconds, while in the 2U group, the same bonding agent was re-applied and light activated for 20 seconds.

To simulate the provisional phase of restoration, the specimens were bonded to an acrylic rod (4 mm in diameter x 3 mm in height) with non-eugenol temporary cement (TempBond® NE, Kerr, Orange, CA, USA) under 10 N load. The excess cement was mechanically cleaned before setting. The bonded specimens were kept in distilled water at 37 °C. After 7 days, the acrylic rod was detached and temporary cement was removed using a spoon excavator and the tooth was polished with pumice slurry on a rotary brush.

Self-adhesive resin cement (RelyX™ U200, 3M ESPE, Minneapolis, MN, USA) was used for the final cementation composite rods (4 mm in diameter x 3 mm in height) to the tooth specimens under 10 N load in all experimental groups. The excess cement was removed with dental instruments followed by light polymerization for 20 seconds, twice. The bonded specimens were stored in distilled water at 37 °C for 24 hours and processed for the cyclic loading test.

The tooth specimens were placed on cyclic loading mounting stubs with self-cure acrylic resin. The stubs were transferred to testing chamber and held with screws. A round-end stainless steel antagonist (6 mm in diameter) was used to apply the 50 N load with 2 Hz cycle for 50,000 cycles to the composite rod perpendicular to the bonded interface at room temperature.

After the cyclic loading procedure, all specimens were processed for the marginal leakage testing by applying nail varnish (Revlon®) to the entire tooth surface of the

specimens, except 1 mm from the bonding interface, then immersed in 50% silver nitrate solution in a dark chamber at 37 °C for 24 hours. To reduce diamine silver ion to metallic silver grains, the specimens were rinsed thoroughly with water and immersed in photo-developing solution and exposed to light from a flood lamp for 8 hours. Each tested specimen was cut in half perpendicularly to the bond surface using a low-speed diamond saw (Buehler®, Lake Bluff, IL, USA). The cut surfaces on both sides were polished with 1000-grit silicon carbide abrasive papers, ultrasonically cleaned, and air dried. One half was examined under a stereomicroscope (Olympus Corp., Tokyo, Japan). Leakage scores were calculated as a percentage of silver nitrate penetration in length along the resin-dentin interface compared to the total length of the cut surface.

The other half was processed for μ TBS testing by further sectioning to produce 1 x 1 mm² beams using a low-speed cutting machine under water cooling. Two beams from each specimen, totaling 10 beams per group, were selected to test μ TBS using a universal testing machine (Universal Testing Machine, Lloyd Instruments, UK). A cyanoacrylate adhesive (Model Repair II Blue, Dentsply) was used for attaching the beam to the gripping device. The load was applied to the specimens with a constant crosshead speed of 1 mm/min until failure. The maximum failure load (N) was recorded and converted to stress units (MPa).

The failure modes were examined under stereomicroscope and a digital camera (SZX7 & SZ2-ILST LED illuminator stand & E-330, Olympus, Tokyo, Japan) at 50x magnification and classified into adhesive failure (A), cohesive failure (C), or mixed failure (M). Dentin at the bond interface was partially decalcified in 6 mol/L of HCl solution for 25 seconds, then deproteinized with 6% NaOCl solution for 3 minutes. All debris from the surface was removed by immersing the specimens in ultrasonic cleanser for 5 minutes. Each specimen was examined at bond interface under scanning electron microscope (SEM) at 1,000x magnification (JSM-IT300, Joel, Peabody, MA, USA).

One-way ANOVA was used for comparing the μ TBS of direct bonding technique and the two IDS techniques (one layer and two layers) of

the two adhesive systems (three-step etch-and-rinse and self-etch universal systems). In addition, paired t-test was used for comparing the μ TBS between the different techniques of the same bonding adhesive system.

Results

The results from one-way ANOVA analysis suggested that IDS technique using both dental adhesive systems (1TE and 1U) provided significantly higher μ TBS (23.56±3.26, 21.51±1.30 MPa) than direct bonding technique (13.56±2.74 MPa) ($p < 0.001$). However, there was no significant difference among the 1TE and 1U groups (Figure 1).

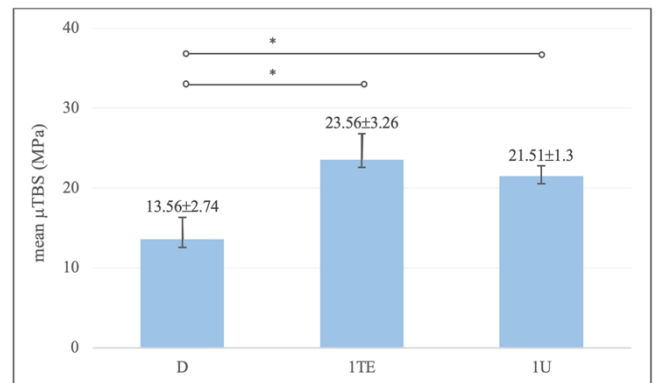


Figure 1. Mean±SD microtensile bond strength (μ TBS) values in MPa for direct bonding and the one-layer IDS techniques of different adhesive systems applied to dentin.

Abbreviations: IDS, immediate dentin sealing.
*Indicates the significant difference ($p < 0.05$).

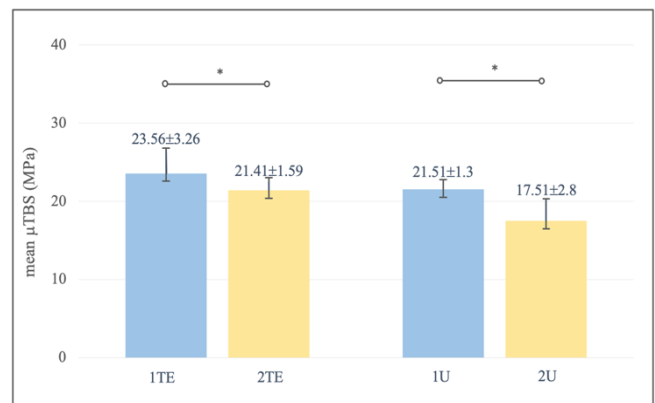


Figure 2. Mean±SD microtensile bond strength (μ TBS) values in MPa for the one-layer and two-layer IDS techniques using etch-and-rinse and universal adhesive systems.

Abbreviations: IDS, immediate dentin sealing.
*Indicates the significant difference ($p < 0.05$).

A significant reduction of μ TBS was observed when using the two-layer IDS technique compared with the one-layer IDS techniques using paired t-test (Figure 2). The μ TBS of 2TE (21.41 ± 1.59 MPa) was significantly lower than those of 1TE (23.56 ± 3.26 MPa) ($p < 0.01$). Similarly, the μ TBS of 2U (17.51 ± 2.80 MPa) was also significantly lower than 1U (21.51 ± 1.30 MPa) ($p < 0.01$).

The direct bonding technique showed the highest marginal leakage value (1.78 ± 0.56 mm), followed by the one-layer IDS techniques of 1U (1.13 ± 0.73 mm) and 1TE (1.1 ± 0.40 mm). There was a slight increase, but no significant difference in marginal leakage for both of the two-layer IDS techniques, 2TE (1.26 ± 0.21 mm) and 2U (1.20 ± 0.48 mm).

In the SEM images, all three bonding techniques showed different thicknesses of hybrid layer. The direct bonding technique showed the least thickness of the adhesive layer. Among the one-layer IDS techniques, the 1TE group showed a thickness of hybrid layer comparable with the 1U group, but the longer resin tags were found (Figure 3). The thickness of the adhesive layer of the two-layer IDS techniques, in both the 2TE and 2U groups, was obviously thicker than that of the one-layer IDS techniques of both dental adhesives (1TE and 1U). The resin tags were found in both 1TE and 2TE groups.

The mixed failure was the majority found in all groups (60% in the D and 1TE groups, and 70% in the 2TE, 1U, and 2U groups) followed by cohesive failure (20%-30%) in the D, 1U and 2U groups, and adhesive failure (30%) in the 1TE and 2TE groups.

Discussion

Application of IDS techniques after tooth preparation in this study provided significantly greater bond strength of resin cement¹³⁻¹⁵, but this was in disagreement with findings of some studies in which the bond strength of IDS groups was not significantly different to a freshly prepared tooth surface as in direct bonding technique³. The possible explanation was that the dental adhesives, etch-and-rinse and universal adhesives, applied on freshly cut dentin have a better demineralization capacity, which provide a more complete hybrid layer than self-adhesive resin cement¹⁶. Using either three-step etch-and-rinse or self-etch universal adhesive systems as the one-layer IDS technique showed no significant difference in μ TBS values. This result was not consistent with the results of Rujirakanusorn's study¹⁴, which showed that etch-and-rinse adhesive provided significantly higher μ TBS than universal adhesive. The results of IDS technique using different adhesive systems is still controversial as several studies showed no different results^{17,18}, whereas some suggested that etch-and-rinse adhesive systems provided better results^{14,19}.

The use of two-layer IDS techniques using both adhesive systems in this study did not improve the microtensile bond strength significantly, which agreed with the study of Elkassas et al.²⁰. This was probably due to the

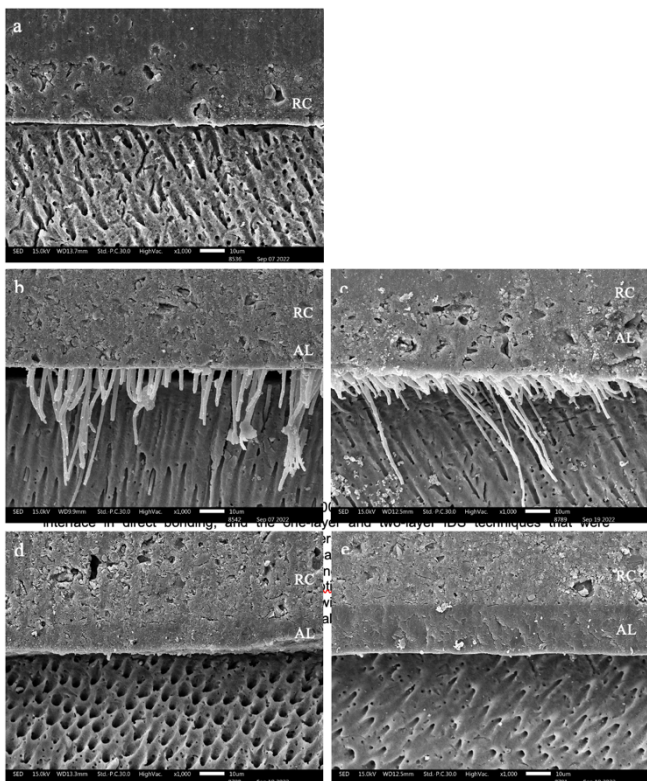


Figure 3. Representative SEM images at 1,000x magnification of the resin cement-dentin interface in direct bonding, and the one-layer and two-layer IDS techniques that were performed with the etch-and-rinse and universal adhesive systems: a. D sample, b. 1TE sample, c. 2TE sample, d. 1U sample, e. 2U sample.

Abbreviations: AL, adhesive layer; D, direct bonding; IDS, immediate dentin sealing; RC, resin cement; 1TE, one-layer IDS technique with OptiBond™ FL; 2TE, two-layer IDS technique with OptiBond™ FL; 1U, one-layer IDS technique with 3M™ Single Bond Universal; 2U, two-layer IDS technique with 3M™ Single Bond Universal.

increased thickness of the additional layers of adhesives, particularly when Single Bond Universal was used. The thickness rose from 7.5 μm to 430 μm without affecting the thickness of hybrid layer¹². However, the maximum tensile bond strength was obtained when the thickness of the adhesive was between 7.5 to 25 μm ¹². The excessive thickness over 45 μm acted as a weak point, since the point of failure was transferred from hybrid layer to junction between hybrid layer and adhesive resin, resulting in a reduction of the μTBS value^{12,21}.

The higher values of the μTBS test in all IDS groups (1TE, 1U, 2TE, and 2U groups) compared with direct bonding technique corresponded with the characteristics found in the SEM images, which showed the thicker hybrid layer. However, the μTBS values of the 1TE, 1U, 2TE, and 2U groups were not significantly different. When the SEM was assessed, both the 1TE and 2TE groups showed thicker hybrid layers and longer resin tags interlocked with dentinal tubules in the SEM images, while the 1U and 2U groups showed thinner hybrid layers and no resin tags were found. Instead of using strong acid such as phosphoric acid, mild acidic primer in the universal adhesive system^{22,23} can remove less mineral content from dentin surface²⁴ and partially dissolve smear layer and smear plug occluded in the dentinal tubules²⁴⁻²⁸, whereas phosphoric acid in TE groups can completely remove smear layer and allow more resin penetration into collagen network of decalcified dentin to form thicker hybrid layers and into dentinal tubules to form longer resin tags²⁹.

Although the U groups showed thinner hybrid layers, the comparison of the μTBS value to the TE groups supported the evidence that universal adhesives can form chemical bonds to the primed dentin³⁰. The Single Bond Universal used in this study has three main ingredients: Vitrebond™ copolymer, MDP monomer, and silane, which are responsible for chemical interaction with dentin. The acidic MDP monomer has been proved to provide an effective chemical bond to dentin by forming a stable nano-layer at adhesive interface and a stable MDP-Ca salt deposition yielding high bond stability³¹. This mild acid has the advantage over the strong phosphoric acid that no water rinsing is needed after etching procedure, resulting in less

technique sensitivity and less post-operative sensitivity^{25,32}.

The cyclic loading process, which simulated the clinical situation in this study, provided no differences of μTBS between the 1TE, 2TE, 1U and 2U groups. Every adhesive system could undergo plastic deformation and micro-gaps at resin-dentin interface due to the repeated stress applied to the restorations. Therefore, the μTBS values after cyclic loading decreased^{33,34}; consequently, all values in both the TE and U groups were comparable.

In all groups, the failure mode analysis showed a high incidence of mixed failure, failure within dental adhesive and bonding interface^{35,36}. Lower levels of incidence were found in adhesive failure, failure at the interface between adhesive and restoration material, and cohesive failure, which is failure in either tooth substance or composite resin^{28,37}.

The cyclic loading process has been claimed to not only introduce porosity formation at resin-dentin interface^{38,39}, but also increase marginal leakages of adhesive layers. All tested groups showed no significant difference of marginal leakage after the cyclic loading test. These results were not consistent with the study by Hashimoto et al.,⁸ which found that multiple coatings of adhesive could reduce nanoleakage and improve the quality of resin-dentin bonds.

The one-layer and two-layer IDS groups showed no significantly different results in terms of μTBS and marginal leakage. After the first layer of adhesive was polymerized, it provided a complete hybrid layer over dentin surface and was less likely to allow any resin infiltration into the un-infiltrated matrix, resulting in improvement of the quality of the adhesive layer. Therefore, the use of the double application of dental adhesive for IDS techniques would not be necessary⁴⁰.

Conclusions

The two-layer IDS techniques using both three-step etch-and-rinse and self-etch universal adhesive did not show any advantage in both the μTBS test and marginal leakage over the one-layer IDS technique. Moreover, the one-layer IDS techniques showed significantly greater μTBS than direct bonding technique.

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Declaration of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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