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Original Article

Effect of Pulpal Pressure and Immediate or Delayed Dentin Sealing Technique on Microtensile Bond Strength of an Adhesive Resin Cement to Dentin: An *In Vitro* Study

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Abstract

Objectives: To evaluate the effect of pulpal pressure and immediate (IDS) or delayed dentin sealing (DDS) technique on microtensile bond strength (μTBS) of an adhesive resin cement to exposed human dentin.

Methods: Fifty extracted human third molars were divided into 2 groups: pulpal and non-pulpal pressure (PP, NP), which represented vital and non-vital tooth. Each group was divided into 5 subgroups: direct restoration (control), DDS technique and 3 IDS techniques, [etch-and-rinse (TE), self-etching (SE) and universal adhesive (U)]. The hydrostatic pressure of 20 cmH_2O was applied prior to adhesive application in PP groups during the experiment. The self-adhesive resin cement was used to bond a composite disc onto dentin for all groups. Ten small square beams from each group were fabricated and collected for μTBS testing and mode of failure monitoring.

Results: All three IDS groups yielded a significantly higher μTBS than control and DDS groups in both pulpal conditions. DDS groups yielded the lowest μTBS. The presence of pulpal pressure caused a significant reduction of μTBS in TE and DDS groups.

Conclusions: The IDS technique could produce higher μTBS than the DDS and direct restoration technique in both pulpal conditions.

Keywords: pulpal pressure, dental adhesives, immediate dentin sealing

Introduction

Magne *et al.*(1-3) first introduced the immediate dentin sealing (IDS) technique, which seals freshly prepared dentin with dental adhesive to improve the success of indirect bonded restorations; for example inlays, onlays, veneers and other fixed prostheses. The immediate application of dental adhesive after tooth preparation helps prevent any contamination and compression of decalcified matrix of dentin caused by the cementation of indirect restorations. The dental adhesive also improves bond strength, increases retention, and reduces marginal leakage of the restorations.(2,4-8)

The technique of resin coating on dentin after tooth preparation in fixed prosthodontic work was first recommended by Pashley and colleagues[9] to prevent microleakage during provisional period. Many *in vitro* studies[2-6,10-12] also reported that this technique produced high tensile bond strength and improved shear bond strength. However, most bond strength studies[2,3,5,6] were done in dry tooth specimens without consideration of pulpal pressure, which produces continuous outward flow of fluid after dentin is exposed in vital tooth.[13,14] The slightly positive pressure (approximately 20 cmH_2O)[14-17] in dental pulp generates this flow and resists the invasion of bacteria and its toxin. However, it may interfere the penetration of a dental adhesive into decalcified matrix to form a hybrid layer, resulting in impaired bonding of the restorations.[18]

Since the results from literatures of dental adhesive administrative techniques are still inconclusive, little is known about the influence of the presence of pulpal pressure on bond strength of dental adhesives used in immediate dentin sealing technique. This study aimed to evaluate the effect pulpal pressure and immediate or delayed dentin sealing (DDS) technique on microtensile bond strength of resin cement and exposed dentin in extracted human teeth. The three dental adhesive systems used in IDS technique was also investigated.

Materials and Methods

Fifty sound extracted human third molars were collected under informed consent of each patient. The use of human tissue was approved by the Human Experimentation Committee No.74/2019. The samples were stored in 0.5% chloramine-T trihydrate solution for 7 days and then in distilled water grade 3 at 4°C until required, according to ISO/TS 11405/015 specification.[19]

The whole tooth was embedded in epoxy resin with buccal surface facing upward. The root was sectioned off at 1 mm beneath cementoenamel junction (CEJ). The remaining pulp tissue was removed through the opening by forceps. The buccal surface was removed 1.5 mm to expose the dentin using a slow-speed diamond saw (Isomet® 1000, Buehler, Lake Bluff, IL, USA) with water cooling. The cut surface was polished with 400-grit silicon carbide paper under running water for 30 seconds to create a smear layer.

The specimens were randomly assigned into two groups equally. In the presence of pulpal pressure (PP) group, a hydrostatic pressure device, composed of a plexiglass plate (15 mm×15 mm×5 mm) with an 18-gauge stainless steel needle stuck through, was attached to the tooth specimen. The pulpal chamber and the whole system were filled with distilled water and hydrostatic pressure was set at 20 cmH_2O throughout the experiment. Care was taken to avoid air bubbles in the system. The specimen of non-pulpal pressure (NP) group was left unconnected.

Each of the pulpal condition groups were divided further into five subgroups: direct restoration as control (con), delayed dentin sealing technique (DDS), immediate dentin sealing technique, which includes three-steps etch-and-rinse (TE), two-steps self-etch (SE), and universal adhesive (U).

One of the three dental adhesive systems (TE, SE, and U) was randomly applied to the freshly prepared dentin surface as IDS technique, according to the manufacture's direction. In the TE groups, the dentin was treated with 37% phosphoric acid for 15 seconds followed by rinsing with running water for 20 seconds and air-dried. The primer was applied on the etched surface with micro-brush for 15 seconds and gently blown with air. The surface was painted with a bonding agent, followed by gentle airflow and light activated for 30 seconds. In the SE groups, the primer was painted on dentin for 20 seconds and air blown. The bonding agent was applied, thinned with gently air blown and light activated for 20 seconds. The U groups consisted of an application of one bottle adhesive on the dentin surface for 20 seconds, followed by air-drying and light activated for 20 seconds.

Provisional cement (Temp-Bond™ NE, KERR, Brea, CA, USA) was used to bond an acrylic disc (4 mm diameter, 4 mm thick) onto the prepared dentin surface of DDS, TE, SE and U subgroups under a constant load of 10 N until completely set. The specimen was stored in distilled water for 1 week. The acrylic disc and provisional cement were removed and the dentin surface was cleaned using spoon excavator and a rubber cup containing water slurry pumice with a slow speed contra-angle hand-piece. In the control group, direct restoration technique, the prepared dentin surface was exposed only to resin cement.

In the permanent cementation process, a composite disc (4 mm diameter, 4 mm thick) was cemented on dentin surface with resin cement (RelyX™ U200, 3M ESPE, Minneapolis, MN, USA) in all subgroups under a constant load of 10 N, any excess cement was removed by a micro-brush before applying light activation for 20 seconds. The specimen was stored in distilled water at 37°C for 24 hours before microtensile bond strength testing.

Four vertical grooves and four horizontal grooves 1 mm apart were sectioned perpendicularly to the bonding surface using a slow speed cutting machine to produce small square beams. Two beams were selected from each specimen, which made up ten beams for each subgroup for μTBS test. Each beam was attached to the gripping device of the universal testing machine (Instron Corp., Canton, MA, USA) using cyanoacrylate adhesive (Model repair II blue, Tokyo, Japan). The tensile load was applied with a constant crosshead speed of 0.5 mm/min until failure. The actual bonded area (mm^2) of each tested beam was measured with a pincer-type digital caliper. The microtensile bond strength (MPa) was calculated from a maximum force (N) divided by bonded area (mm^2). The data was analyzed using 2-way ANOVA to investigate the interaction between the presence of pulpal pressure and the bonding techniques and Tukey multiple comparison.

The failure modes of all specimens were analyzed using a stereomicroscope system and digital camera (SZX7 & SZ2-ILST LED illuminator stand & E-330 & Olympus, Tokyo, Japan) at 50x magnification and were then classified into adhesive failure (A), cohesive failure (C) or mixed failure (M). One sample from each group was cut longitudinally through the bonding interface. The cut surface was decalcified by immersing in 6 mol/L of HCl solution for 25 seconds and followed by deproteinizing with 6% NaOCl solution for 3 minutes. All debris was cleaned from surface using ultrasonic cleanser. The specimen was processed for examination in more detail under scanning electron microscope (SEM) (JSM-5910LV, Joel, Peabody, MA, USA).

Results

Two-way ANOVA statistical analysis suggested that there was a significant interaction ($p<0.05$) among the bonding techniques and the presence of pulpal pressure. Therefore, the simple main effect analysis of the two main factors was carried out to examine the effect of one main effect at different levels of the other main effect. Overall, μTBS of non-pulpal pressure group (25.76±4.93) was greater ($p<0.05$) than that of present pulpal pressure group (23.94±5.18 MPa). Within the bonding techniques, the DDS group showed the lowest μTBS (18.09.19±3.79) followed by control group (21.57±2.42). All IDS groups (TE, SE, U) showed significantly higher μTBS than DDS and control groups. Within the IDS group, μTBS in TE group (29.74±2.52) was the highest, but not significantly different from those in SE and U groups (27.89±1.37, 27.17±2.92). The microtensile bond strength (means and standard deviation) from all groups is presented in Table 1.

Table 1: Mean±SD of microtensile bond strengths obtained from 5 bonding techniques under non-pulpal pressure and presence of pulpal pressure conditions.

Bonding technique	n	Mean±SD (MPa)	
		Non-pulpal pressure (NP)	Presence of pulpal pressure (PP)
Control (con)	10	21.98±2.27[b]	21.15±2.62[b]
Delayed dentin sealing technique (DDS)	10	20.19±4.09[b]	15.99±1.96[a]
IDS technique with Optibond™ FL (TE)	10	31.25±2.64[d]	28.67±1.09[c]
IDS technique with Clearfil™ SE bond (SE)	10	27.85±1.61[c]	27.57±1.27[c]
IDS technique with 3M™ Single bond universal (U)	10	27.55±2.91[c]	26.80±3.03[c]

Different letters indicated the significant different (p<0.05)

A closer examination of non-pulpal pressure groups, all IDS-NP groups showed a significantly higher μTBS than DDS-NP and control-NP groups. The TE-NP group (31.25±2.64) had a significantly higher μTBS value ($p<0.05$) than SE-NP and U-NP groups (27.85±1.61, 27.55±2.91). However, there was no significant difference among control-NP (21.98±2.27) and DDS-NP (20.19±4.09) groups.

Under the present pulpal pressure condition, the μTBS of all IDS-PP groups were significantly higher than those of DDS-PP and control-PP groups. The control-PP (21.15±2.62) group had a significantly higher μTBS ($p<0.05$) than DDS-PP (15.99±1.96) group, while there was no significant difference among TE-PP (28.67±1.09), SE-PP (27.57±1.27) and U-PP (26.80±3.03) groups. In a comparison between non-pulpal pressure and present pulpal pressure condition, the μTBS of TE-PP or DDS-PP group (28.67±1.09, 15.99±1.96) was significantly lower than TE-NP or DDS-NP (31.25±2.64, 20.19±4.09) with $p<0.05$.

Under stereo-microscope examination, the percentage of failure mode of each group under both pulpal conditions were compared. Mixed failure was found in a majority of IDS groups (75% in TE, 70% in SE, 70% in U), while DDS and control groups had high proportion in adhesive failure (65%, 60%) (Table 2). SEM images showed complete adhesive layer at the bonding interface in all IDS groups, but not in DDS and control. For both pulpal conditions, TE and U groups showed thicker adhesive layer, whereas TE and SE groups presented longer resin tag (Figure 1).

Discussion

IDS technique provided higher μTBS than DDS and direct restoration techniques in both pulpal pressure conditions in this study, which was consistent to other studies.[2-6,20] The results indicated that immediate application of dental adhesive on freshly prepared dentin, IDS technique, has more advantages than application after provisional cementation (DDS technique) or no adhesive application (direct restoration). IDS technique not only

Table 2: Percentage of failure mode.

Group	% Failure Mode		
	Adhesive	Mixed	Cohesive
Control (con)			
Non-pulpal pressure	60	40	0
Presence of pulpal pressure	70	30	0
Delayed dentin sealing technique (DDS)			
Non-pulpal pressure	60	40	0
Presence of pulpal pressure	80	20	0
IDS technique with Optibond™ FL (TE)			
Non-pulpal pressure	10	80	10
Presence of pulpal pressure	20	70	10
IDS technique with Clearfil™ SE bond (SE)			
Non-pulpal pressure	20	70	10
Presence of pulpal pressure	30	70	0
IDS technique with 3M™ Single bond universal (U)			
Non-pulpal pressure	20	70	10
Presence of pulpal pressure	30	70	0

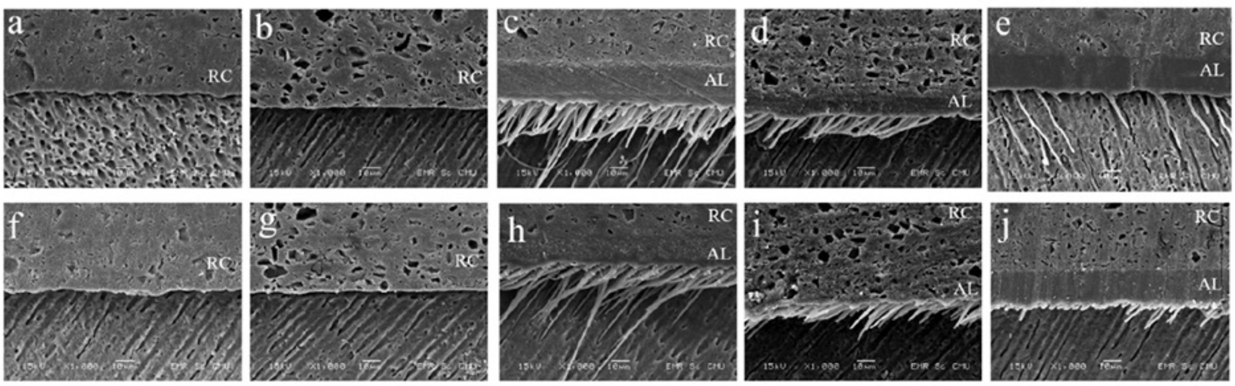

Figure 1: SEM micrograph at 1,000x magnification of resin cement-dentin interface under non-pulpal pressure condition (a-e) and presence of pulpal pressure condition (f-j); a. con-NP sample b. DDS-NP sample c. TE-NP sample d. SE-NP sample e. U-NP sample f. control-PP sample g. DDS-PP sample h. TE-PP sample i. SE-PP sample j. U-PP sample Abbreviation: RC: resin cement, AL: adhesive layer, con: control, DDS: Delayed dentin sealing, TE: IDS technique with Optibond™ FL, SE: IDS technique with Clearfil™ SE bond, U: IDS technique with 3M™Single bond universal, NP: Non-pulpal pressure, PP: Presence of pulpal pressure

improve bond strength of permanent resin cement, but also has less post-operative dentin sensitivity.[19,21]

Contamination of provisional cement after applying immediate dentin sealing seemed to have little effect on bond strength of resin cement.[6] Magne and colleagues[3] suggested that oxygen-inhibited layer of methacrylate group in dental adhesive remained active up to 12 weeks and could interact chemically with permanent luting cement even though it was contaminated by provisional cementation.[22]

The reduction of the μTBS in every group with simulated pulpal pressure suggests that outward flow of dentinal fluid interfered with the bonding of adhesive to a certain degree.[18,23-25] DDS-PP and TE-PP groups had significantly lower μTBS than DDS-NP and TE-NP groups. Although self-adhesive resin cement required some water to release hydrogen ion from acidic monomer to demineralize dentin[26], the outward fluid flow seemed to dilute acidic monomer[27,28], resulting in an impaired smear layer and lower capacity of resin cement infiltration.[29] Moreover, the dentin surface in DDS group was contaminated from provisional cement[6,30,31], which affected the μTBS.

In the etch-and-rinse adhesive system, TE group, although μTBS of TE-PP was significantly lower than TE-NP, they still showed the highest values in both pulpal conditions. Strong phosphoric acid used in TE group could remove smear layer and smear plug more completely than mild acidic monomer in SE and U groups.[32] In non-pulpal condition, hydrophilic primer could re-expand the collapsed demineralized collagen matrix and allowed hydrophobic adhesive to diffuse in and form a complete hybrid layer effectively. The reduction of μTBS suggests that the penetration of primer and adhesive was retarded in this study, which contrasted to many studies.[14,33]

Means μTBS in SE and U groups were compared in both pulpal conditions, indicated that pulpal pressure did not affect the bond strength. Acidic monomer of these two bonding systems has advantages over phosphoric acid in TE group, since water rinsing was not required.[34] A 10-methacryloxyldecyl dihydrogen phosphate (MDP), as acidic monomer, interacts with hydroxyapatite in dentin and enamel chemically, which allows adhesive to form stronger phase nano-layer at bonding interface and results in high bond stability and high mechanical strength.[35] Moreover, as a mild acid, it partially removes smear layer and leaves the deeper part of smear plug in the tubules, which limits dentin permeability.[36] As a result, bonding adhesive is allowed to penetrate the demineralized layer effectively under pulpal pressure condition[35] This study ascertained that no more advantage was found when polyalkenoic acid copolymer was added to 3M™ Single bond universal in U group than in SE group. Polyalkenoic acid is claimed to increase hydrophilicity of the adhesive, which improves bonding stability between dentin and the adhesive.[37,38] On the contrary, some studies[39,40] have disputed that this copolymer might compete MDP in binding the hydroxyapatite and then prevent monomer to approach the polymerization site due to its high molecular weight.

Under simulated pulpal pressure condition, the IDS technique, using three dental adhesives, showed a higher μTBS than direct restoration, which corresponded to the other studies.[10,36] The result of this study supported the use of IDS technique in vital tooth in order to reduce dentin sensitivity during provisional cementation period and also to increase bond strength of luting cement. Among three adhesive systems used in the IDS technique, although they yield a comparable bond strength, self-etch adhesive and universal bonding system have advantages over etch-and-rinse system since they have less technique sensitive and produce less post-operative sensitivity.[32] On the other hand, without pulpal pressure in non-vital tooth, the etch-and-rinse adhesive system used in IDS technique provided the highest μTBS.

Conclusions

All immediate dentin sealing techniques produced higher μTBS than delayed dentin sealing technique while etch-and-rinse system yielded the highest μTBS, although it was affected by pulpal pressure. The μTBS obtained when self-etch or universal adhesive system was used was not affected by the presence of pulpal pressure.

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