

# Effect of double layer immediate dentin sealing technique on dentin permeability and microtensile bond strength

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**Objectives:** To examine the effect of one-layer and two-layer Immediate dentin sealing (IDS) treatment on dentin permeability and microtensile bond strength ( $\mu$ TBS) of dentin and resin cement under simulated pulpal conditions.

**Materials and Methods:** Thirty extracted third molar teeth were cleaned, disinfected, and embedded in a resin block. The buccal surface was exposed to dentin, and the block was connected to a hydrostatic pressure device set at 20 cmH<sub>2</sub>O. The teeth were divided equally into three groups: NoIDS; no dentin treatment before direct cementation, 1IDS; a single layer of dental adhesive was applied, and 2IDS; a second layer was applied in addition to the first layer. Each group was further divided in half to perform the permeability test and  $\mu$ TBS test. For permeability test, the replica techniques captured droplets from the dentinal tubules, then investigated under a Scanning Electron Microscope. The size and total volume of droplets were measured and calculated. For  $\mu$ TBS test, 1IDS and 2IDS groups were contaminated with temporary cement, and then all groups were cemented with resin cement. The bonded samples were cut into small beams to conduct the  $\mu$ TBS test. Data analysis was performed using one-way ANOVA and Tukey's multiple comparisons. Additionally, the correlation between  $\mu$ TBS and total fluid volume was analyzed using Pearson correlation.

**Results:** The NoIDS group exhibited larger diameter droplets with irregular shapes, while the 1IDS group showed smaller droplets in higher numbers. No droplets were observed in the 2IDS group. In contrast, the bond strength was significantly higher in the 2IDS group, followed by the 1IDS group and the NoIDS group, respectively. There was a negative correlation between  $\mu$ TBS and total fluid volume.

**Conclusion:** Single-layer IDS technique reduced dentin permeability, whereas two-layer IDS effectively blocked dentin permeability, resulting in increased  $\mu$ TBS.

**Keywords:** bond strength, dentin-bonding agents, dental cement, dentin permeability, hydrostatic pressure

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## Introduction

For indirect restoration, such as crowns and bridges, tooth preparation usually involves the removal of a certain amount of dentin, which increases the risk of pulpal inflammation and post-operative sensitivity. This procedure could injure the odontoblast and adjacent cells at the superficial level of pulp tissue [1]. Additionally, bacteria and their toxins can invade the underlying pulp through

the exposed dentin surface [2]. In vital pulp, there is a spontaneous outward flow of dentinal fluid that counteracts this invasion and interferes with the infiltration of dental adhesive, resulting in the challenge of forming a complete hybrid layer for maximum strength [3-6].

The application of dental adhesive onto freshly-prepared dentin after tooth preparation, known as the immediate dentin sealing (IDS) technique, was introduced to reduce post-operative

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sensitivity as well as improve the bond strength of restorations, especially in fixed prostheses [7-8]. This sealing also prevents the contamination of provisional cement on dentin, which might reduce the bond strength [9]. The original IDS technique, proposed by Magne and colleagues [8], involved applying a single layer of dental adhesive on the freshly cut dentin. Although this technique claimed to seal the dentinal tubules and improve bond strength, certain cases of post-operative sensitivity still arose in clinical situations, which resulted from incomplete sealing of the dentinal tubules because the outward flow of dentinal fluid interferes with the resin infiltration.

To ensure the complete seal of dentinal tubules and achieve higher bond strength, some studies have explored the use of additional layers of dental adhesive, known as the multilayer IDS technique [10-12]. Hashimoto [10] and Ito *et al.* [11] found that increasing the thickness of dental adhesive used in the IDS technique could improve dentin sealing and the bond strength of resin cement. Moreover, the additional adhesive layer could reduce marginal leakage by forming a uniform thickness and reducing the oxygen inhibited layer. However, it is important to limit the thickness since an excessively thick adhesive layer can initiate adhesive failure [11]. Although the use of a thicker layer of adhesive appeared to be beneficial, the effect of adhesive thickness on the sealing ability and bond strength of resin cement has not been thoroughly investigated.

Most *in vitro* studies [7, 13-15] reported a higher bond strength of dental adhesives compared to *in vivo* studies since they did not consider the pulpal pressure that generated an outward flow of dentinal fluid. This fluid flow can interfere with bonding penetration, resulting in lower bond strength [16]. In a vital tooth, the pressure within the pulp is higher than the atmospheric pressure, causing fluid to emerge on

the dentin surface, which can be recorded by impression and replica techniques [17].

Based on the previous information, the experimental hypothesis is that the application of a double layer of dental adhesive in the IDS technique may lead to a reduction in dentin permeability, while simultaneously increasing the microtensile bond strength ( $\mu$ TBS) between dentin and resin cement. The objectives of this study were to investigate the effect of one-layer and two-layer IDS treatment on dentin permeability and  $\mu$ TBS of dentin and resin cement under simulated pulpal conditions. This information will be valuable for enhancing the IDS technique and optimizing clinical outcomes for patients undergoing indirect restorations.

## Materials and Methods

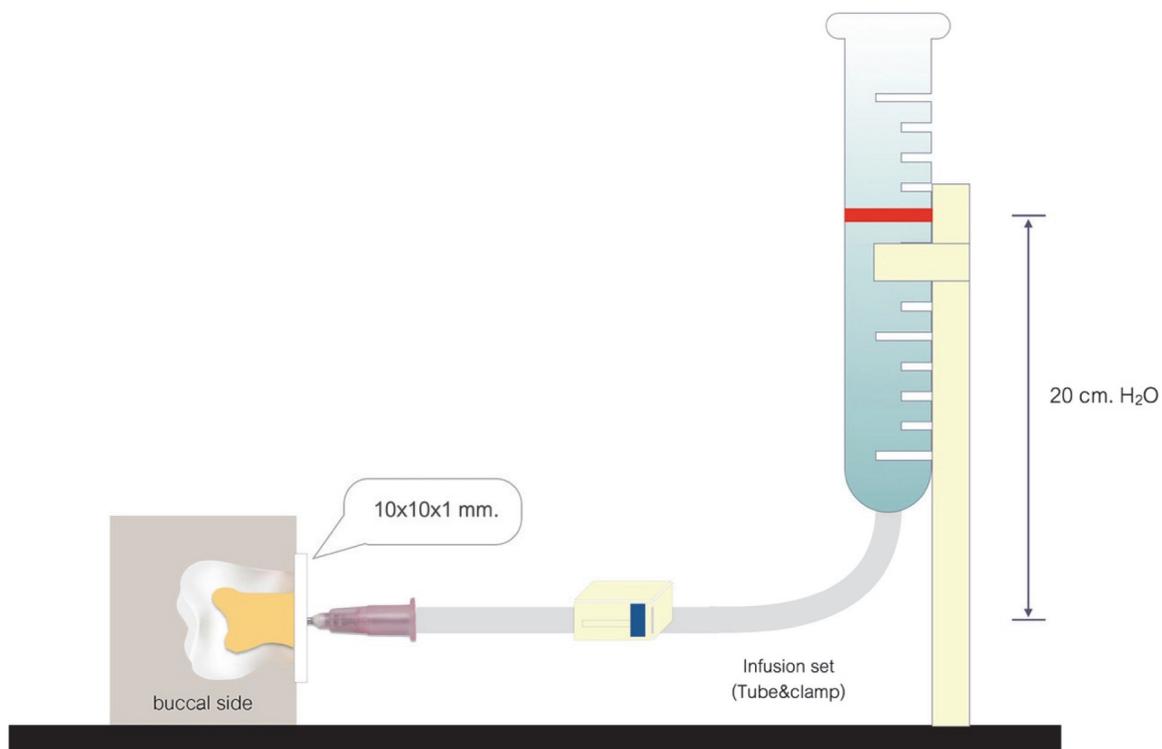
The use of human tissue in this study was approved by the Human Experimentation Committee Faculty of Dentistry Chiang Mai University No. 54/2021. Thirty intact third molar teeth, which were caries-free and non-restored, were collected after extraction for use in the study. They were cleaned and disinfected in 0.1% chloramine T solution for up to 7 days and stored in grade 3 distilled water at 37 °C for a maximum of 6 months according to ISO/TS 11405/2015.

The tooth samples were horizontally embedded in epoxy resin, with the buccal surface left uncovered. The enamel on the buccal surface was removed using a diamond wafer blade on a low-speed cutting machine under water coolant (Isomet 1000 precision low-speed saw, Buehler, Illinois, USA) at 450 rpm and 150 N. After the initial exposure of dentin, an additional 1 mm of dentin was removed using the same technique to create a large, flat dentin surface followed by polishing the cut surface with 400 grit silicon carbide abrasive paper under water for 10 seconds.

To simulate normal human physiological pulpal pressure, a value of 20 cmH<sub>2</sub>O was used [18]. The root of each tooth was cut off 2 mm below the cemento-enamel junction, and the remaining pulp tissue was removed through the cut opening. An acrylic sheet (10x10 mm), with an 18-gauge stainless steel tube inserted, was attached to the apical cut end using cyanoacrylate glue. Subsequently, a hydrostatic pressure device was connected to the pulp chamber of each experimental tooth. The hydrostatic pressure was maintained at 20 cmH<sub>2</sub>O throughout the experiment (Figure 1).

Thirty teeth were divided equally into three groups. Group I comprised direct cementation (NoIDS) using self-adhesive resin cement without dentin sealing, while Group II was one-layer IDS

(1IDS) using the Clearfil SE Bond (Clearfil™ SE Bond, Kuraray CO., LTD., Osaka, JAPAN) Primer applied immediately on the prepared dentin with a light brushing motion. The primer was left for 20 seconds and then dried gently, followed by the application of Clearfil SE bonding agent and airflow, after which the adhesive was cured with light intensity of 550-650 mW/cm<sup>2</sup> for 20 seconds. Group III comprised two-layer IDS (2IDS) with the first layer of Clearfil SE Bond Primer and bonding agent applied in the same way as Group II, followed by the application of an additional layer of the same bonding agent. The specimens in each group were equally divided to monitor the ultrastructure change on the treated dentin surface and conduct the  $\mu$ TBS test.



**Figure 1** Simulated hydrostatic pulpal pressure application system. The water level that determined pulpal pressure was set at 20 cmH<sub>2</sub>O above atmospheric pressure.

### Permeability test

The treated dentin surface under simulated pulpal pressure was recorded using the impression and replica technique, and further examined under a scanning electron microscope (SEM). The dentin surface was dried with a cotton pellet and left for 30 seconds before the application of a small volume of condensation silicone impression material (Xantopren VL plus<sup>®</sup>, Henry Schein, Inc., Northern Ireland, UK) onto the prepared surface, which was allowed 5 minutes for setting. The polyether material (Impregum<sup>™</sup> Soft Light Body Polyether, 3M, Minnesota, USA) was injected into the initial impression to create a replica of the recorded dentin surface. The replica was processed and examined under a scanning electron microscope at a magnification of 500x (JSM-IT300 InTouchScope<sup>™</sup> Scanning Electron Microscope, JEOL Ltd., Tokyo, JAPAN). SEM images were captured to calculate the volume of the fluid droplet.

The SEM images were analyzed using the ImageJ program (ImageJ, National Institutes of Health, Maryland, USA) to determine the volume of fluid droplets. Each droplet was measured for its diameter in two perpendicular directions, and the diameters were divided by two to obtain the radii ("a" and "b"). For circular droplets, where "a" is equal to "b", this value was also used to represent the height of the droplets ("c"). It was assumed that these droplets formed hemispheres, so this height value was used to represent the height of all droplets, irrespective of any compression caused by the impression material. Therefore, the use of low-viscosity impression material in minimal quantities was important for recording the shape of a droplet to limit the weight of the material and allow the fluid droplet to form. Assumptions were made in order to measure the fluid volume and facilitate comparisons within this study. Individual circular droplets were treated as hemispheres and the formula  $\frac{4}{3}\pi\frac{r^3}{2}$  was used to calculate the volume.

In the case of elliptical shape droplets,  $\frac{4}{3}\pi\frac{abc}{2}$  was used to calculate their volume. When multiple droplets merged, a circular or elliptical shape was drawn to facilitate the volume calculation [19].

### Microtensile bond strength test

For groups II and III, the dentin surfaces were contaminated with temporary cementation before  $\mu$ TBS testing. The acrylic rod (Unifast<sup>™</sup> Trad, GC America Inc., Illinois, USA) (5 mm diameter and 3 mm height) was bonded onto the treated dentin surface with temporary cement (TempBond<sup>®</sup> NE, Kerr Corporation, California, USA) to simulate the provisional cementation procedure. After storing specimens in distilled water for 7 days and removing the acrylic rods, the bonded surfaces were cleaned and polished with pumice and distilled water using a rubber cup attached to a low-speed handpiece for 10 seconds. The tooth was rinsed and dried before final cementation with resin cement.

The composite rod (3M<sup>™</sup> Filtek<sup>™</sup> Z350 XT Universal Restorative, 3M, Minnesota, USA) (5 mm in diameter and 3 mm in height) was bonded to the treated surface with self-adhesive resin cement (RelyX<sup>™</sup> U200, 3M, Minnesota, USA) in all groups. To control the position of the bonding and the thickness of the resin cement, a 5 mm hole aluminum foil with a thickness of 25  $\mu$ m was used. Resin cement was mixed according to the manufacturer's instructions, and the composite resin rod was fixed onto the prepared dentin with a 10-N load applied for 60 seconds. The excess cement was removed using an explorer before curing with an LED curing light for 20 seconds on each side. The bonded specimens from each group were sectioned into slabs using a diamond wafer blade on a low-speed cutting machine at 450 rpm and 150 N under water cooling with the section plane perpendicular to the adhesive-dentin interface. Each slab was further sectioned

into beams with an approximate area of 1 mm<sup>2</sup>. Twenty-five beams were randomly selected from each group (n=25). Each beam was fixed to the grips of a Universal Testing Machine (6800 SERIES SYSTEM RETROFITS, INSTRON<sup>®</sup>, Massachusetts, USA) using cyanoacrylate adhesive (Model Repair II Blue, Dentsply-Sankin, Tochigi, JAPAN). The test was conducted in tension, with a cross-head speed of 1 mm/min until a fracture occurred. The bonded area of each beam (mm<sup>2</sup>) was measured using a pincer-type digital caliper. The maximum tensile load was calculated by dividing the maximum force (N) by the bonded area (mm<sup>2</sup>), resulting in units of stress (MPa).

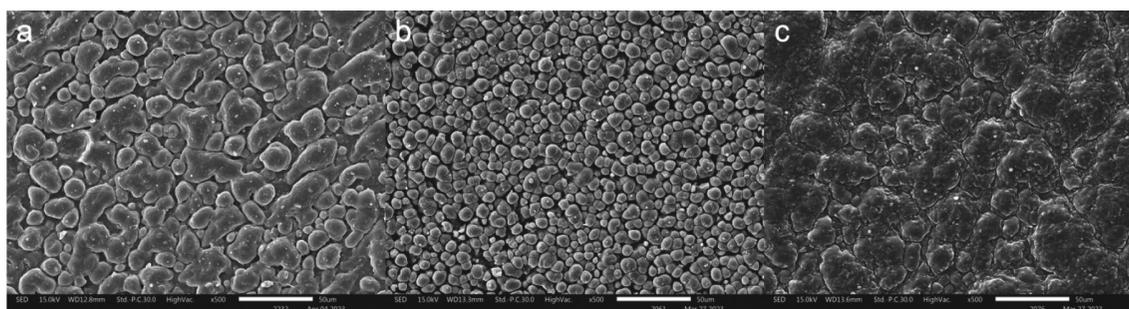
The failure modes of all specimens were analyzed using a Stereo Microscope system and digital camera (SZX7 & SZ2-ILST LED illuminator stand & E-330 & Olympus, Olympus, Tokyo, JAPAN) at 50x magnification. Subsequently, they were classified into adhesive failure, cohesive failure, or mixed failure.

The SPSS program version 25.0 (SPSS<sup>®</sup> Statistics, IBM, New York, USA) was used to analyze the data. The  $\mu$ TBS values were tested for normality and homogeneity of variances.

Subsequently, the  $\mu$ TBS among the experimental groups were compared using one-way ANOVA and Tukey's multiple comparisons, with a significance level of 0.05. The correlation between droplet volume and  $\mu$ TBS was calculated using Bivariate correlation (Pearson correlation) and then visualized through a scatter diagram to demonstrate the correlation.

## Results

SEM images of the replicas revealed fluid droplets on the dentin surface of the NoIDS and 1IDS groups, while no fluid droplet was found on the dentin surface of the 2IDS group. The droplets on the dentin surface treated with the one-layer IDS technique were smaller in diameter but larger in number compared to the droplets on the freshly prepared dentin surface (NoIDS) (Figure 2). In the NoIDS group, the individual droplet diameter and total droplet volume were significantly greater, but the droplet count was lower than in the 1IDS group (Table 1), indicating that smaller droplets merged to form larger droplets.



**Figure 2** Scanning electron micrographs at 500x magnification of the replicas taken from (a) NoIDS, (b) 1IDS, and (c) 2IDS under simulated pulpal pressure conditions. (a) Non-homogeneous shape of droplets merging together, greater in size, and lower in number. (b) Homogeneous shape of circular droplets, smaller in size, and greater in number. (c) No fluid droplets were presented.

$\mu$ TBS values were explored for normality using tests to assess data with normal distribution. The result showed normality in terms of kurtosis, skewness, boxplot, histogram, and Q-Q plot. Additionally, the data showed significance in homogeneity of variances. One-way ANOVA with Tukey's multiple comparisons tests indicated that the  $\mu$ TBS in the 2IDS group was significantly higher ( $p < 0.05$ ) compared to both the 1IDS and noIDS groups. Additionally, the 1IDS group was also significantly higher compared to the NoIDS group, as shown in Table 1.

Under stereo-microscope examination, the percentage of failure modes was compared for each group. A majority of the 1IDS and 2IDS groups exhibited mixed failure, while the NoIDS group had a higher proportion of adhesive failure (Table 2).

The correlation coefficient obtained from the calculation is -0.308, indicating a negative correlation between these two factors. This negative correlation is illustrated in the form of a scatter diagram (Figure 3), where the linear trend in the graph represents the strength of the negative correlation, as indicated by the correlation coefficient value. The correlation analysis further confirmed a negative relationship between the volume of fluid emerging on the dentin surface and the  $\mu$ TBS with a trend that  $\mu$ TBS was low in the groups with high total droplets volume. Specifically, the NoIDS group exhibited lower  $\mu$ TBS compared to the 1IDS group. Additionally, the 2IDS group, which had no droplets detected, showed a higher  $\mu$ TBS.

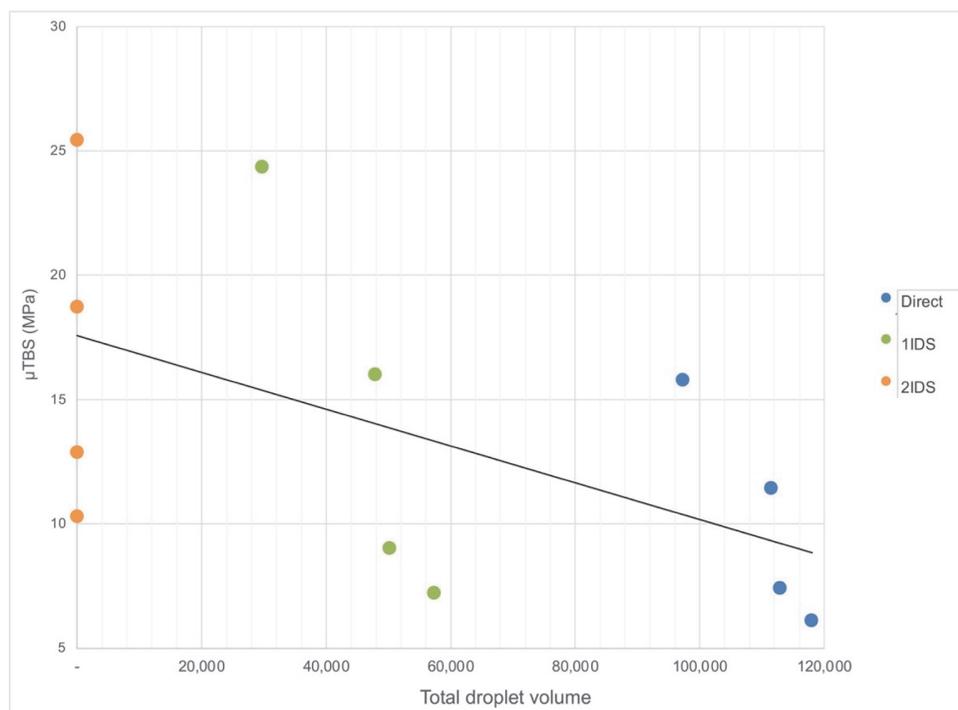
**Table 1** Mean  $\pm$ SD of the number of droplets count, individual droplet diameter, total droplet volume, and  $\mu$ TBS of resin cement and dentin from NoIDS, 1IDS, and 2IDS groups

Group	Droplet count (n)	Individual Droplet diameter ( $\mu$ m)	Total Droplet volume ( $\mu$ m <sup>3</sup> )	Microtensile bond strength (MPa)
NoIDS	261.50 $\pm$ 85.99 <sup>a</sup>	11.56 $\pm$ 4.25 <sup>a</sup>	112,178.60 $\pm$ 8,854.15 <sup>a</sup>	9.45 $\pm$ 1.92 <sup>a</sup>
1IDS	925.00 $\pm$ 169.67 <sup>b</sup>	5.85 $\pm$ 1.77 <sup>b</sup>	49,003.60 $\pm$ 11,720.43 <sup>b</sup>	12.85 $\pm$ 4.81 <sup>b</sup>
2IDS	0 <sup>c</sup>	0 <sup>c</sup>	0 <sup>c</sup>	15.98 $\pm$ 4.03 <sup>c</sup>

Different letters in each column identify significant differences ( $p < 0.05$ )

**Table 2** Percentage of failure mode observed from specimens after performed  $\mu$ TBS test in NoIDS, 1IDS, and 2IDS groups.

Group	% Failure Mode		
	Adhesive	Mixed	Cohesive
NoIDS	80	20	0
1IDS	44	56	0
2IDS	24	76	0



**Figure 3** The scatter diagram showed a negative correlation between total droplet volume and  $\mu$ TBS, with each color representing a sample from a specific group and a linear trend to show the correlation.

## Discussion

The study challenges the null hypothesis that there is no difference in the permeability of dentin after applying one-layer or two-layers dental adhesive. Additionally, it also challenges the null hypothesis that there is no difference in microtensile bond strength between dentin and resin cement after applying one-layer or two-layers of dental adhesive in the IDS technique. The results of this study lead to the rejection of both null hypotheses

The impression and replica technique was confirmed to be effective for recording fluid droplets on dentin surfaces, particularly for visualizing changes after various surface treatments with desensitizing agents and dental adhesives [20]. This study provided evidence to

explain how  $\mu$ TBS improved following the application of dental adhesive using the IDS technique. Under simulated pulpal pressure, mimicking conditions of the vital pulp, larger and irregularly shaped fluid droplets were observed emerging from the dentin surface. These large droplets resulted from the merging of small droplets. This finding was similar to those reported by Rangcharoen [21]. Following the application of a single layer of dental adhesive, a significant decrease in the size of individual droplets was observed, accompanied by increases in the number of fluid droplets, which was consistent with the observations made by Dusadeedumkoeng [20]. The application of two layers of dental adhesive resulted in the absence of clearly visualized droplets, indicating complete blockage of the outward flow of dentinal fluid [5, 22].

The impression material used in this study, Xantopren VL plus<sup>®</sup>, has been employed extensively in previous studies [17, 20, 21, 23, 24] for recording fluid exudation on the dentin surface. This slow-setting and low-viscosity polyvinyl siloxane (PVS) impression material allows for a set time of approximately 6-8 minutes [25]. Its slightly hydrophilic properties, characterized by a higher water contact angle [26], facilitate the imprinting and replication of fluid droplets on the dentin surface for examination under SEM. Additionally, the impression and replica technique provides a snapshot of the dentin surface at a specific point in time, lacking a continuous record of fluid dynamics [5, 17, 27].

The presence of a smear layer and smear plugs on freshly prepared dentin, although they can retard the outward flow of dentinal fluid, did not completely block it. As a result, fluid droplets could still be observed on the dentin surface, and they tended to have larger diameters and volumes. The application of the one-layer IDS technique created an additional obstacle that can further reduce dentin permeability and the volume of dentinal fluid passing through [28].

The importance of simulating pulpal pressure in studying the bond strength of dental adhesives is that the presence of outward flow can interfere with the bonding process and potentially reduce the bond strength of the material. Comparing the results obtained without simulated pulpal pressure, the  $\mu$ TBS of several adhesive systems are significantly lower than  $\mu$ TBS of the total etched system, while tested under simulated pulpal pressure conditions the  $\mu$ TBS of all adhesive systems are not significantly different [15, 29, 30].

The dentinal fluid was able to penetrate through the dental adhesive layer before it completely polymerized, forming micro-channels that allowed fluid to pass through. However,

no droplets were observed in the two-layer IDS group, providing visual evidence that the double application of dental adhesive completely sealed off the dentin and prevented the outward flow of dentinal fluid. This finding supports the recommendation of applying two layers of dental adhesive to improve the bond strength of the bonding interface by reducing fluid permeability [10, 11]. Not only did the bond strength improve when this micro-channel was completely sealed, but postoperative sensitivity might decrease, enhancing the effectiveness of the dental adhesive.

The results revealed that specimens in the two-layer IDS group exhibited significantly higher  $\mu$ TBS values compared to both the one-layer IDS and direct cementation groups (NoIDS), respectively. These findings are consistent with the results reported in the literature [10, 11], which suggest that increasing the thickness of the dental adhesive contributes to the improvement of bond strength in dental restorations and resin cementation. The double application of dental adhesives, with and without interlayer curing, could increase the adhesive layer's thickness and enhance the bond strength [11, 31]. However, excessive adhesive thickness may have adverse effects, creating weak points and leading to a decrease in  $\mu$ TBS values, compromising the overall bonding performance [10, 11, 32]. The double application of dental adhesive might provide a more suitable thickness for the adhesive resin to improve bond strength and could potentially prevent postoperative sensitivity for both direct composite fillings and the final cementation of fixed prostheses. However, it is important to note that the pulpal simulation used in this study may not entirely replicate the conditions of the natural human tooth pulp and the intraoral environment.

## Conclusion

Under simulated pulpal pressure conditions, fluid droplets were observed on the dentin surface of prepared tooth samples. The application of the one-layer IDS technique reduced dentin permeability, while the two-layer IDS technique completely sealed the exposed dentin and prevented fluid penetration, resulting in a significant increase in  $\mu$ TBS.

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