

# The fractographic analysis of three dentin bonding agents on tooth surface

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## ABSTRACT

The dentin bonding agent is hydrophilic resin that can strongly bind to dentin surface, both in chemical and physical-mechanical ways. The dentin surface is good for the resin bonding when the surface is in moist condition. Three types of dentin bonding agents: Voco, Prime & Bond NT and Excite were used in this research and their application methods are called as total-etched technique. The objective of this research is to examine the difference of tensile bond strength of the three bonding agents on the moist dentin surface. Bovine incisivus teeth were cut and sharpened using diamond bur then smoothed with sandpaper. Dentin surfaces were etched with 37% phosphoric acid, washed with 20 cc aquadest, and dried with blot-dry technique. The preparation teeth were inserted into desiccator with minimum humidity 60% and maximum 90% for one hour. After removed from the desiccator, the Voco agent was applied on the teeth in first group, and then followed by the Prime & Bond NT and Excite agents, respectively. The resulting sample was stored within the room temperature. After 24 hours, the tensile bond strength was tested using Autograph instrument. The results indicated that the tensile bond strength of Voco and Prime & Bond NT agents were higher than Excite both at humidity 60% and 90% ( $p \leq 0.05$ ). In conclusion, the dentin bonding agents with acetone solvents have a higher tensile bond strength compared with those with alcohol solvents.

Key words: dentin bonding agents, dentin collagen, blot-dry technique, fractographic analysis

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## INTRODUCTION

The dentin bonding agents are widely used in the operative dentistry, particularly as materials for treating the class V cavity. This is because gingival tissues with the increasing ages will physiologically experience a retraction so that their dentins and cementum will be fairly open. One proper option to restore this condition is using a composite resin restoration.<sup>1,2,3</sup> As the combined bonding between the dentin and composite resin, the dentin bonding agents, notably the hydrophilic resins that can strongly bind to the moist dentin tissues will be used here.<sup>4,5</sup>

Contrary to the enamel, the wet environment in the dentin is mainly caused by a presence of the fluids in dentin tubule. The deeper cavities in the dentin produce an elevated number of dentin tubules making this region wetter. In some areas next to the pulp, the number of tubules decrease from  $\pm 45,000/\text{mm}^2$  to  $\pm 20,000/\text{mm}^2$  in several regions close to dentin-enamel junction.<sup>2,6</sup>

Previous researches suggested that chemical compounds which could be used as the dentin bonding agents were including HEMA (2-hydroxyethyl methacrylate), BPDm (bisphenyl dimethacrylate), 4-META (methacryloyloxyethyl trimellitate anhydride) and other agents. However, HEMA is the most common used agent because it has advantageous chemical-physical properties, stable

enough since preservative agents are added, such as hydroquinone, and its construction is inexpensive.<sup>7-11</sup>

The HEMA-based dentin bonding agents on the dentin surface may take the form of either chemical or mechanical bonding. In the first place, the chemical bonding occurs when HEMA carbonyl groups interact with the dentin collagen amino groups which in turn generate amide or peptide bonds.<sup>12,13</sup> Conversely, the mechanical bonding can be explained below. In three-dimensional sense, the dentin collagen represents a network or braid of the fibril collagen. There are nano cavities between the fibrils into which the HEMA agents will penetrate and undergo polymerization. The HEMA solidifying within the interfibrillar cavities constitute a mechanical anchoring/retention of the dentin bonding agents.<sup>14,15</sup> In general, the success of the HEMA-based dentin bonding on the collagen is generally dependent on several factors, such as low monomer viscosity, type and concentration of monomer, the conditioning acid application, temperature and humidity around the collagen.<sup>6,8,16</sup> According to Craig *et al.*<sup>4</sup> and Swift *et al.*,<sup>17</sup> some dentin bonding agents contain multi-functional monomers (primer and adhesives) where hydrophilic groups are useful for reaching adequate wetting and penetrative properties. The hydrophobic properties will polymerize with and bind to composite resin on them. The solvent agents usually used are acetone, alcohol and

water.

Furthermore, collagen tissues existing in the dentin are type I collagen.<sup>18</sup> Collagen usually used as material in the research is a sequence of amino acids, proline, proline, glycine {HN-(pro-pro-gly)<sub>5</sub>-COOH}.<sup>19,20</sup> According to Breschi *et al.*,<sup>16</sup> the fibril collagen length is about  $\pm$  0.5–1,0  $\mu$ m, mayor fibril diameter 60–80 nm, minor branch diameter 10–25 nm and interfibrillar cavity 15–20 nm.

In this research, the moist dentin surface was made consistent with the previous researches.<sup>21–24</sup> The researchers argued that the moist surface was correlated with a water content or humidity surrounding the dentin. Hence, our analysis was carried out based on the minimum humidity 60% and maximum humidity 90% at temperature 25 °C. The humidity is defined as a condition related to water content existing around the material.<sup>22,23</sup> Moreover, the fractographic analysis put into execution in the research represented “a tensile strength test” imposed on composite resin/dentin bonding agents until they were dissociated from the dental surface and some researchers called this technique as microtensile bond test.<sup>25,26</sup>

The purpose of this research is to analyze fractographically the three types of the HEMA-based dentin bonding agents using moist-milieu approach.

## MATERIALS AND METHODS

The materials used in the research are bovine incisivus teeth (obtained from the slaughterhouse in Pegirian Surabaya); three types of the HEMA-based dentin bonding agents: Voco (Germany), Prime & Bond NT (Dentsply-Caulk, Germany) and Excite (Ivoclar-Vivadent, Schaan/Liechtenstein); acid etching solution (Ivoclar-Vivadent), self-curing acrylic: Vertex (Dentimex, Holland). The ingredients of the three dentin bonding agents are shown in table 1.

**Table 1.** Ingredients of the dentin bonding agents

Material	Ingredients
Voco	Bis-GMA, HEMA, butylated hydroxyl toluene (BHT), acetone, organic acid
Prime & Bond NT	Urethane dimethacrylate dipentacrylthiol pentacrylate phosphate, polymerizable dimethacrylate, acetone
Excite	HEMA, dimethacrylate, phosphoric acid acrylate, silicone dioxyde, alcohol

Instruments used in the research are diamond disk, diamond bur, sandpaper no. 400 and 1000 (Fuji Star, Japan), desiccator with vacuum-tap, united thermometer and

hygrometer (Haar-Synth, Hygro, Germany), compressor/air suction (Schuco, USA), Autograph AG-10 TE (Shimadzu, Japan).

The methods in this research is similar to the previous researches.<sup>21</sup> The preparation teeth were cleaned carefully and gently by removing debris present on the teeth surface using brush, while for soft or hard tissues using sharp scalpel. During the cleaning process, the teeth should always be in wet condition. Then the teeth were cut using diamond disk and embedded firmly on the dental stone cylinder block. The dentin should face forward. For preparation up to the dentin part, diamond bur was required. The dentin surface was smoothen using silicone sandpaper no. 400 and proceeded with no.1000. The dentin was covered with adhesive tape (isolation) 3 mm in diameter and attached just in the middle of the surface. In the next stage, the dentin specimens were primed or smeared with 37% phosphoric acid etching agent using cotton pellets for 15 seconds, then washed gently with 20 cc aquadest from injection sput and dried by wiping gently using cotton pellets. In subsequent stage, the preparation teeth were put into desiccator for one hour under minimum humidity 60% and maximum humidity 90%.

When removed immediately from the desiccator, the teeth were primed with primary solution and bonding agents that have been thoroughly mixed (Voco) as described shortly below. Firstly, the solution was dropped slightly on disposable brush and primed/smeared on the dentin surface and stand for 30 seconds and irradiated with light curing unit for 20 seconds (the method was carried out in accordance with the manufacturer’s direction). The cylinder block was inserted into plunger. The opposite plunger was filled with *self-cured* acrylic as the solidifying material on the dentin bonding.

For solidification with dentin bonding agents of Prime & Bond NT and Excite, their application was the same as Voco. All resulting samples were kept in the room at  $\pm$  28 °C for 24 hours.

After 24 hours, the tensile bond strength test was carried out using Autograph instrument (at Airlangga Joint Laboratory). When in use, the instrument was operated with following provisions: cross-head speed = 10 mm/minute, operational (range) level: 5, load cell capacity = 5 kN/500 kgf. The results as seen on the display have kgf unit. The test dentin surface area is about 7.1 mm<sup>2</sup>. Subsequently, the data collected were then analyzed using One-Way ANOVA test at  $\alpha$  = 0.05, and proceeded with Turkey HSD test.

## RESULTS

The tensile bond strength of three dentin bonding agents, means and standard deviation are showed in table 2.

From table 2 it appeared that the tensile strength of Voco at humidity 60% was higher than at 90%. In addition, the t test results indicated that p value = 0.001 (p < 0.05), indicating that there was a significant difference between

the Voco's tensile strengths at humidity 60% and 90%. To know whether the Voco's tensile strength was normal or not, the tensile strength was tested using Kolmogorov-Smirnov technique. The test results showed that at humidity 60%,  $p$  value = 0.916 ( $p > 0.05$ ) and at humidity 90%,  $p$  value = 0.560 ( $p < 0.05$ ). This indicates that the two trial groups are normal.

**Table 2.** The tensile bond strength of three dentin bonding agents on the dentin surface at minimum and maximum humidity (MPa)

Dentin bonding agents	Means ( $\bar{X}$ ) and Standard Deviation (SD)		n
	Humidity 60% (minimum)	Humidity 90% (maximum)	
	Voco	16.61 ± (1.96)	
Prime & Bond NT	18.01 ± (1.71)	10.67 ± (2.13)	8
Excite	13.10 ± (2.05)	7.01 ± (1.98)	8

Along similar lines, the tensile bond strength of Prime and Bond NT at humidity 60% was higher than at humidity 90%. The  $t$  test was carried out to know whether there was a difference between the two trial groups. The test results demonstrated that there was a significant difference between the two trial groups in associated with the tensile bond strength ( $p < 0.05$ ). Likewise, The results of the Kolmogorov-Smirnov test suggested that Prime & Bond NT trial group possessed normal data distribution (at humidity 60%,  $p$  value = 0.988  $> 0.05$ , while for humidity 90%,  $p$  value = 0.539  $> 0.05$ ).

Similarly, the tensile bond strength of Excite at humidity 60% was statistically significant higher compared with that at humidity 90% ( $p = 0.001 < 0.05$ ). The data distribution in the two trial groups both at humidity 60% and 90% were normal ( $p$  value for humidity 60% = 0.746 and  $p$  value for humidity 90% = 0.540).

To prove that the research had homogenous data, the statistical analysis was carried out using Levene test. The test results showed that at humidity 60% the dentin bonding agents with Voco, Prime & Bond NT and Excite brands possessed  $p$  value = 0.686 ( $p > 0.05$ ), meaning that the three agents were homogenous. Similarly, at humidity 90% those agents were also homogenous with  $p$  value = 0.921 ( $p > 0.05$ ).

To know whether there was a difference between overall trial groups the statistical analysis using ANOVA test was carried out. At humidity 60% the significance level of the three dentin bonding agents was 0.001 ( $p < 0.05$ ), suggesting that significant differences existed among all trial groups at humidity 60%. Conversely, at humidity 90% the significance level was 0.004 ( $p < 0.05$ ), indicating that at humidity 90% all trial groups possessed significant

differences. Moreover, to see a difference in each trial group the Tukey HSD test was carried out and the results could be seen in table 3.

**Table 3.** The difference in tensile bond strength of three bonding agents at humidity 60%

Dentin bonding agents		Significance Level
Voco	Prime & Bond NT	0.329
	Excite	0.004*
Prime & Bond NT	Voco	0.329
	Excite	0.000*
Excite	Voco	0.004*
	Prime & Bond NT	0.000*

Note: \*): significant difference at  $\alpha = 0.05$

When tensile strengths of Voco and Prime & Bond NT were compared, there was no statistically significant difference at humidity 60% ( $p > 0.05$ ). On the other side, there was statistically significant difference between Voco and Excite groups in line with their tensile strengths ( $p < 0.05$ ). In comparison between Prime & Bond NT and Excite groups, there was statistically significant difference ( $p < 0.05$ ).

**Table 4.** The difference in tensile bond strength of three bonding agents at humidity 90%

Dentin bonding agents		Significance Level
Voco	Prime & Bond NT	0.806
	Excite	0.022*
Prime & Bond NT	Voco	0.806
	Excite	0.005*
Excite	Voco	0.022*
	Prime & Bond NT	0.005*

Note: \*): significant difference at  $\alpha = 0.05$

The difference in tensile strengths at humidity 90% can be seen in table 4. The tensile bond strength between the trial groups was the same at humidity 60% and 90%. There were statistically significant differences ( $p < 0.05$ ) between Voco and Prime & Bond NT and Excite groups when they were compared. On the other hand, there was no significant difference ( $p > 0.05$ ) between Voco and Prime & Bond NT.

## DISCUSSION

It has been commonly argued that optimal tensile bond strength of the HEMA-based bonding agents on the dentin surface was reached in moist condition.<sup>2,3</sup> Summitt *et al.*<sup>2</sup> argue that the dentin bonding agents (HEMA) can bind sufficiently to the fibril collagen when the dentin surface

is in moist condition instead of the dry or wet surface. This may be true since when the dentin surface is dry, the fibril collagen will collapse. Consequently, the dentin bonding agents have difficulty in binding to those collapsed collagen. On the contrary, when the surface is too wet, more water molecules exist around the fibrils thereby inhibiting the bonding agents penetrative power to bind to the fibril collagen. Therefore, the moist environment in the dentin surface plays a considerable role in the bonding or the environment of the dentin surface must have an optimal and proper humidity.<sup>6</sup> The results of this research have proved that humidity 60% is an optimal humidity since the tensile strengths in Voco, Prime & Bond NT and Excite agents are significantly higher than the trial groups at humidity 90%. This is because fibril collagen tissues are greatly permeable and active at humidity 60% thereby making it easier for them to bind to the dentin bonding agents both chemically and mechanically.<sup>2,6</sup> In chemical bonds, a bonds between resin agents and collagen will generate strong amide bonds, namely interatomic primer bond/covalent bond.<sup>12,14</sup> In addition, there were hydrogen bonds, interfunctional group bonds between both compounds, including hydroxyl group, carboxylate, amino and carbonyl, and a complex reactions happened between dentin calcium ions and resin bonding agents. On the other hand, the mechanical bonds may occur because the bonding agents penetrate into dentin tubules, on irregularly dentin surfaces (microscopically forming slit, pore, crack and undercut). The mechanical bonds may also occur due to a penetration of the bonding agents into nano interfibrillar cavities of the collagen network. These exposed fibrils are not merely collagen tissues but also enclosed by several proteins, such as non-collagen protein and proteoglycan. Both proteins have good wetting properties so that when adhesive monomers will make a contact with fibrils, they must compete with water molecules on the protein surfaces.

The results showed that tensile strengths in the Voco and Prime & Bond NT agents were significantly higher than Excite agents both at humidity 60% and 90% ( $p \leq 0.05$ ). This could be explained by the fact that the Voco and Prime & Bond NT were equipped with acetone solvent while Excite with alcohol solvent. As we have known that acetone possesses higher vaporousness and water chasing effect compared with alcohol. Thus, when primed or smeared on the dentin the acetone will quickly vaporize and prevent water molecules from dentin surface and allow many resin bonding molecules to bind to the fibril collagen. It is also noted that acetone is able to dilute resin bonding solution which in turn reduces resin viscosity. The low resin viscosity produces a good wetting on the dentin surface, leading to an increased surface energy and ultimately enhances resin tensile strength on the dentin.<sup>27</sup> However, the researcher said that too high acetone concentration could cause hybrid layers to undergo a crack and the strain strength of the resin would decline. The maximum acetone concentration is about 37% of the weight.

Summitt *et al.*<sup>2</sup> and Anusavice<sup>3</sup> proposed that an

adequate tensile bond strength between the dentin bonding agents and the dentin surface was much dependent on adequate wetting properties of the bonding agents that brought about a small contact angles between the two agents. Thus, in order to wet the dentin surfaces evenly and completely, the resin viscosity should be low. In addition, the bonding agent's capability to wet the surface (wettability) is mainly influenced by several factors. For example, a cleanliness of the agent surface to which the dentin surface is attached and oxide layers in the surface may inhibit the bonding, including organic fluids. However, the acid etching on the dentin could increase the wetting and surface roughness, and may cause the opening of the dentin tubule.<sup>28</sup> The proper wetting procedure will result in a good joining between the resin agents and fibril collagen. But until today there is a scant knowledge about the bonding monomer affinity against the dry or wet fibril collagen. Therefore, it was widely stated that the resin agents could bind to the dentin surface even though its affinity was fairly low.<sup>6</sup>

Anusavice<sup>3</sup> and Craig *et al.*<sup>4</sup> explained that when a contact angle between the adhesive fluids and the solid surface at an interface was small, the adhesive molecules could adhere strongly to the agent molecules. This means that the wettability of the adhesive material is good enough. On the contrary, if the contact angle is large the wetting capability will decrease accordingly.

Additionally, both interface surfaces must be able to attract one another to allow the adhesion occurrence. This condition can occur without considering the second phase of the substance whether it is solid, liquid or gas. The energy on surface of the substance is usually larger than inside. This is because of the geometrical lattice pattern for its molecules. That is, the molecule lattices in all atoms attract one another in balanced fashion inside the substance. The increased energy for each area unit on the surface is intimately correlated with the surface energy or surface tension.<sup>2,3,5</sup>

Regarding the tensile bond strength on the dentin surface, some factors we should consider are those which may cause a failure in bonding, including, firstly, whether a fluoride has ever been applied in the teeth. This can reduce the wetting properties of the resin agents. Second, the presence of smear layer on the tooth surface is likely to decrease the bond strength. Third, the tooth composition may be not homogenous; and finally, the tooth surface may be contaminated by saliva or blood. Again, these factors are likely to produce a bonding failure.

Similarly, the acidity degree or pH of the resin agent solution generally also affected the bond strength on the dentin surface.<sup>13</sup> It was commonly argued that when a dissociation of carboxylic acid or amino groups were inhibited, the hydrogen bond between resin and collagen would improve. In fact, this condition can boost the bond strength. This usually occurs at pH 2. However, when pH increases from 6.6 to 9.0, the tensile strength will decline sharply. This is due to the deformation in hydrogen bond

and functional groups from carboxylic acid undergoing dissociation. The Voco solution used in the research has pH 2, while Prime & Bond NT 1.5 and Excite 1.5. Thus, there is no significant difference in pH of the three bonding agents.

The water content within the dentin is very vital for the presence of the physical and chemical properties of collagen.<sup>6,29</sup> According to them, the presence of water molecules around the collagen generates hydrogen bonds on the fibril collagen or between the fibril collagen. Furthermore, these hydrogen bonds will produce an optimal physical appearance in fibril collagen, making it easier for the fibril to bind to the resin bonding agents.

On the other side, if the water molecules are too small, the hydrogen bonds will dissociate, leading to the fibril collapse, and close contact between the fibrils may happen. Consequently, the bond between peptides is weakened; collagen matrix will wrinkle and being hard so that the collagen is not permeable any longer for the HEMA-based resin bonding agents. With respect to the secondary structure of the collapsed collagen, the amino groups are masked or hidden. Then, the HEMA carbonyl groups have difficulty in binding to the collagen amino groups.

In conclusion, the tensile bond strength of the dentin bonding agents Voco and Prime & Bond NT were significantly higher than Excite. This could be explained by the fact that Voco and Prime & Bond NT were equipped with acetone solvent while Excite with alcohol solvent.

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