

**Evaluation of Bond Strength to Dehydrated Cross-Linked Dentin: A New Perspective**

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**Abstract**

**Background and Objectives:** Dentin is a very complex substrate for bonding as compared to enamel that makes dentin bonding to be more difficult and less predictable. Various attempts have been made in the past to achieve stronger and durable dentin bonds. The objective of the present study is to compare the shear bond strength of acid-etched dentin bonded to wet-bonded, non-cross-linked dentin and dry-bonded, cross-linked dentin.

**Materials and Methods:** 24 extracted human molars were taken for the study. Occlusal enamel and superficial dentin was removed to get flat exposed mid coronal dentin. The specimens were polished with 180-grit silicon carbide paper to create standard smear layer. Flat occlusal surfaces of all the teeth were acid etched for 15secs with

37% phosphoric acid gel and rinsed with water for 60secs. Specimen were then divided into 6 Groups- G1 and G2 without pre-treatment with Grape seed extract (GSE) bonded with wet and dry bonding techniques; G3 and G4 pre-treated with 5 mass% GSE in water and bonded with wet and dry bonding techniques; G5 and G6 pre-treated with 5mass% GSE in ethanol and bonded with wet and dry bonding techniques, respectively. Resin composite build up was done using 3 increments measuring 1.5mm each. After 24hrs, the specimens were subjected to thermal aging procedure for 1 week under 37 °C water bath. After thermo cycling, each specimen was sectioned into two beams of 2x2mm<sup>2</sup> cross-sectional area using a low speed diamond cutter. Shear bond strength (SBS) was determined using a universal testing machine at a

crosshead speed of 1 mm/min. The data obtained from the shear bond strength test was sent for statistical analysis.

**Results:** Dehydrating cross-linked dentin did not lower the shear bond strength when compared to non-cross-linked wet bonded dentin.

**Conclusion:** Cross-linking of acid etched dentin with 5% GSE for 1 minute permits the dentin to be completely air-dried without lowering the bond strength. This prevents the degradation of hybrid layers as seen in wet-bonding and thus can provide clinically stronger and stable resin-dentin bonds.

**Keywords:** Cross-linked, Dry-bonded, Grape seed extract, Shear bond strength, Wet-bonded.

### Introduction

Dentin is a living tissue and constitutes the largest portion of tooth structure. Dentin's microstructure is substantially different from that of enamel. Enamel consists of 92% inorganic hydroxyapatite by volume whereas dentin has only 46% inorganic component. In dentin the hydroxyapatite crystals are randomly arranged in the matrix of collagen fibres.<sup>1</sup> Moreover, dentin has fluid-filled channels called dentinal tubules. The number and diameter of these tubules change with the depth of the dentin. Thus making dentin a very complex substrate for bonding as compared to enamel.<sup>2</sup> The past two decades of research have expanded knowledge of the nature of dentin and the dentin-restorative material interface.

Bonding to dentin require an understanding of the altered morphology of the dentin and its composition after instrumentation. During cavity preparations, dentists expose mineralized tooth dentin that has a modulus of elasticity of 20,000 MPa.<sup>3</sup> By acid-etching dentin they strip away the apatite crystallites in the mineralized matrix to create microporosities in the dentin for resin-infiltration, which solubilizes those crystallites to a depth of 10  $\mu\text{m}$ .<sup>4</sup> After rinsing the acid etched dentin to extract the residual

acid and solubilized minerals, the exposed demineralized dentin have a reduced modulus of elasticity of as low as 3–5 MPa.<sup>5</sup>

As long as the exposed collagen fibrils in the demineralized dentin are suspended in water, they are very pliable. This is because water is one of the strongest hydrogen bonding solvents known<sup>6</sup>, and has a Hoy's solubility parameter for hydrogen(H) bonding cohesive forces of  $40.4 (\text{J}/\text{cm}^2)^{1/2}$  whereas the intrinsic tendency of collagen peptides to form inter-peptide H-bonds with each other in the absence of water is only  $14.8 (\text{J}/\text{cm}^2)^{1/2}$ . Therefore, water prevents the collagen fibrils from forming inter-peptide H-bonds. However, if that water is removed by evaporation or dehydrating solvents, the compliant collagen fibrils rapidly form inter-peptide hydrogen bonds with their nearest neighbours resulting in formation of an impermeable membrane-like structure that prevents the permeation of solvated adhesive monomers around collagen fibrils. This results in resin-dentin bond strength values of only 10 MPa.<sup>7</sup>

So to avoid drying-induced shrinkage, and to create higher resin-dentin bond strengths, Kanca developed what is called the "wet-bonding technique"<sup>8</sup>, where demineralized dentin is allowed to float in 70% water during the monomer infiltration phase of dentin bonding. But there are few concerns regarding this technique.

First is how wet should be the dentin. The goal of resin infiltration during wet-bonding is to replace all of the 70vol% rinse water with 70 vol% adhesive monomers.<sup>4</sup> However, this bonding technique leaves far too much residual water in resin-dentin bonds<sup>9</sup>, providing hydrolytic fuel for the endogenous proteases of dentin matrices which slowly hydrolyse collagen fibrils in resin-bonded dentin, resulting in poor durability of resin-dentin bonds.<sup>4</sup> Further to prevent this, most manufacturers add 30–50 vol% of water-soluble monomethacrylates such as 2-

Hydroxyethyl methacrylate (HEMA) to both scavenge residual water, and act as a solvent for dimethacrylates. However, these monomethacrylates form elastomers that are not cross-linked and attract water to themselves that plasticizes their mechanical properties.<sup>10</sup>

The other problem with wet-bonding is how to remove the excess water. The vapour pressure of pure rinse water at room temperature is 23.8mm of Hg which gets reduced after adding water-soluble adhesive monomers (Raoult's Law).<sup>11</sup> So, by evaporating the rinse water before adding adhesive monomers, it is possible to remove nearly all the rinse water added to dentin within 30 s using a strong, continuous air blast. The other way to get rid of the residual water is "ethanol wet-bonding" as proposed by Tay et al.<sup>12</sup>

Hence in view of eliminating the problems with wet-bonding, this study was done using a cross-linking and dry bonding technique. The inter-molecular cross-linking in collagen fibres is the basis for the stability, tensile strength and viscoelasticity of the collagen fibres. Many synthetic and natural cross-linking agents are therefore used to modify the microstructure of dentin.<sup>13</sup> One of the natural cross-linking agent containing proanthocyanidins is Grape seed extract (GSE) which was used in this study as dentin modifier followed by dry-bonding to compare the bond strength with that of wet-bonding.

## Materials And Methods

### Specimen Preparation

Twenty four molar teeth that were indicated for extraction due to periodontal problems were collected from Department of Oral and Maxillofacial surgery, M.R.Ambedkar Dental College and Hospital Bangalore, Karnataka, India with patients consent. Protocols for infection control as per OSHA and CDC guidelines in collection, storing, sterilization and handling were

followed. Teeth were carefully cleaned and stored in 10% buffered formalin.

### Bonding Procedure

All the specimens were mounted on acrylic blocks. Occlusal enamel and superficial dentin up to thickness of 4mm, were removed with a diamond disc under water coolant. The flat exposed mid-coronal dentin was roughened with wet 180-grit silicon carbide paper to create a standard smear layer. The flat occlusal dentin surface of all the specimens were acid-etched for 15s with 37% phosphoric acid gel. The specimens were then rinsed with water for 60s to remove unreacted acid and to extract solubilized mineral.

Grape Seed Extract (GSE) containing 95% Proanthocyanidins was obtained for preparing the Cross-linking agent. The Cross-linking agent was prepared by mixing:

1. 5mass % GSE in water
2. 5mass% GSE in ethanol

Then the specimens were divided based on the type of bonding into 6 groups:

- GROUP I- Non-Cross-linked, Wet bonded group:  
Specimens were lightly blotted with a tissue. They were left visibly moist when bonding agent (Single Bond Plus; 3M ESPE) was applied in two separate layers, followed by evaporation of the solvent for 5s using a light blow of air by three-way syringe and light-curing for 40s.
- GROUP II- Non-Cross-linked, Dry bonded group:  
The wet dentin surfaces were completely dried for 30s with a continuous air blast at a distance of 10 cm. They were then bonded with Single Bond Plus to the dried dentin surface in similar manner.
- GROUP III- Cross-linked, Wet bonded group:  
Specimens were treated with 5 mass% GSE primer in water for 60s, and were then rinsed for 10s with the appropriate solvent (water). They were then lightly blotted

with a tissue moistened with the same solvent and immediately bonded as in GI.

- GROUP IV- Cross-linked, Dry bonded group:

Specimens were treated with 5 mass% GSE primer in water for 60s, rinsed with air-water spray for 10 s and then air-dried using full strength air from a 3-way syringe at a distance of 10 cm for 30s. They were then immediately dry bonded with Single Bond Plus.

- GROUP V- Cross-linked, Wet bonded group:

Specimens in this group were treated with 5 mass% GSE primer in ethanol for 60s, and were then rinsed for 10s with the appropriate solvent (ethanol). They were then lightly blotted with a tissue moistened with the same solvent and immediately bonded.

- GROUP VI- Cross-linked, Dry bonded group:

Specimens in this group were treated with 5 mass% GSE primer in ethanol water for 60 s, rinsed with air-water spray for 10s and then air-dried using air blast from a 3-way syringe at a distance of 10 cm for 30s. The specimens were then immediately dry bonded with Single Bond Plus. After the bonding procedure, Z100 resin composite (3M ESPE) build-up was done using three increments measuring 1.5mm and were light-cured for 20s each. All the specimens were then subjected to thermal aging procedure for 1 week under 37°C water bath.

### Shear Bond Strength Testing

After thermocycling, each specimen was sectioned into two beams of 2x2mm<sup>2</sup> crosssectional area using a low speed diamond cutter. The beams were then subjected to shear bond strength test using Universal Testing Machine (Mecmesin, IISc, Bangalore) at a cross-head speed of 1 mm/min until the composite cylinder was dislodged from the tooth. Shear bond strength was calculated as the ratio of fracture load and bonding area, expressed in megapascals (MPa). The results were tabulated and subjected to statistical analysis.

### Statistical Analysis

Data entry was done in Microsoft Excel. The values obtained were statistically analyzed using computer software Statistical Package for the Social Sciences (SPSS) (16.0) (SPSS Inc, Chicago, USA). The obtained data was expressed in mean  $\pm$  standard deviation (SD). Mean was compared between different study groups by One Way Analysis of Variance (ANOVA) and multiple comparisons of mean difference between different groups was done using Tukey's Post hoc Analysis. The level of significance [P-Value] was set at  $P < 0.05$ .

### Results

When comparing non-cross-linked groups, specimens in Group I showed significantly higher mean shear bond strength (48.50MPa) than Group II (10.50MPa).

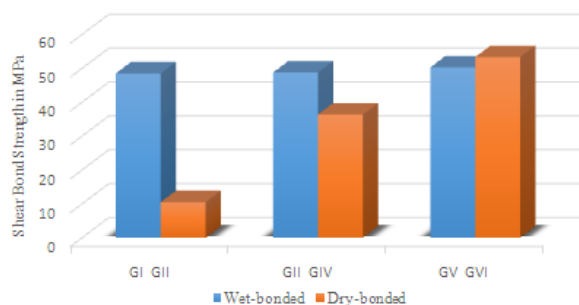
Cross-linked and wet-bonded Group III (48.75MPa) and Group V (50.25MPa) gave similar bond strengths to the non-cross-linked wet bonded Group I (48.50MPa).

But, Group IV (36.35MPa) and Group VI (53.30MPa) that were cross-linked and air dried for 30secs, showed significantly higher bond strengths than the dry bonded non-cross-linked Group II (10.50 MPa).

Among all the groups, Group VI (53.30 MPa) gave the highest bond strength, even after dry-bonding. Though there was no significant difference in bond strength between Group I (48.50MPa) and Group VI (53.30MPa).

Table1- Comparison of mean shear bond strength (in MPa) between different study groups using one-way ANOVA test. Values are mean  $\pm$  standard deviations. Means identified by different superscripts letters are statistically different.

Mean Shear Bond Strength Values		
Groups	Wet Bonding	Dry Bonding
GI and GII (Non-Crosslinked)	GI- 48.50±3.26 <sup>a</sup>	GII- 10.50±3.48 <sup>b</sup>
GIII and GIV ( Cross-linked with 5%GSE in water)	GIII- 48.75±3.92 <sup>a</sup>	GIV- 36.35±4.69 <sup>c</sup>
G V and GVI ( Cross-linked with 5%GSE in ethanol)	GV- 50.25±3.69 <sup>a</sup>	GVI- 53.30±2.93 <sup>a</sup>



Graph 1- Representing mean shear bond strength of different groups

## Discussion

Bonding to enamel has been a routine and reliable aspect of restorative dentistry whereas dentin bonding has proved to be more difficult and less predictable. The reason behind this is the fundamental difference in the nature of the two tissues.

Enamel is largely inert, composed primarily of hydroxyapatite with very low water content. Dentin, on the other hand, is living tissue. Its chemical structure involves both inorganic and organic materials, and it features a highly complex physical structure that varies with the depth of the tissue. And to make it more difficult, the dentin is saturated with oxygen and water. Though water content varies according to the type and depth of

dentin, approximately 13 percent of overall dentinal volume is fluid.<sup>14</sup>

The first principal for dentin bonding was given by Fusayama et al.<sup>15</sup> He claimed that resins could bond to acid etched dentin. Nakabayashi et al.<sup>16</sup> soon confirmed these claims and suggested that improved dentin bonding resulted from resin infiltration within the demineralized dentin surface. The acid etching procedure removes the smear layer, demineralizes the peritubular dentin and produces a collagen mesh on the surface and along tubule surfaces. The curable resin infiltrates the collagen mesh forming a hybrid layer, linking the dentin and the composite together.<sup>17</sup>

Researchers have found that bonding to moist dentin improves the bond strength.<sup>8</sup> The explanation for these findings was that the collagen structure of moist dentin remains open and facilitates primer infiltration. But there are no standard criteria about how moist the dentin should be kept while performing wet-bonding. It has been seen that the residual water provides hydrolytic fuel for the endogenous proteases like Matrix Metalloproteinase (MMPs) and cathepsins which slowly hydrolyse the collagen fibrils in resin-dentin bond, resulting in poor durability of the bond.<sup>4</sup>

In the presence of water, the dimethacrylates undergo phase changes from monomers in solution, to monomers in resin globules suspended in water. Because these resin globules are too large to permeate through the 20nm wide interfibrillar spaces, this results in significant amounts of collagen fibrils in hybrid layers being surrounded by water instead of polymerized resin which leads to hydrolysis of the hybrid layer with time resulting in poor resin-dentin bonds.<sup>18</sup>

Hence to eliminate the problems with wet-bonding, this study was performed. The objective was to compare the



bond strength to cross-linked dentin after dry bonding with that of non-cross-linked dentin after wet bonding.

The inter-molecular cross-linking in collagen fibres is the basis for the stability, tensile strength and viscoelasticity of the collagen fibres.<sup>13</sup> Cross-linking agents are capable of non-specifically cross-linking protein such as collagen and dentin proteases. They have been seen to increase the mechanical properties of dentin collagen and make the fibrils more resistant to degradation.<sup>19</sup>

One of the naturally occurring cross-linking agent is Proanthocyanidin (PCA). Proanthocyanidins belong to category of condensed tannins. They are highly hydroxylated structures capable of forming an insoluble complex with carbohydrates and proteins.<sup>20</sup> PCAs increase collagen synthesis and are potent antimicrobials. Grape seed extract is a natural source of proanthocyanidins. It is a complex mixture of polyphenol monomers, dimers, trimers, tetramers, and polymers<sup>21</sup>. Studies have shown that application of PCAs stabilize the demineralized dentin layer against enzymatic challenges in clinically relevant settings, due to the non-covalent nature of their interaction with collagen molecules.<sup>22</sup>

In the present study, GSE containing 95% proanthocyanidins was used as a cross-linking agent. The idea was to stiffen the dentin with cross-linking agent followed by removal of residual water by air drying for 30 seconds and chemical dehydration using solvents like ethanol. Results of the present study showed that cross-linking and dry-bonding did not lower the resin-dentin bond strength than that of non-cross-linked wet-bonded specimens. The bond strength of specimens treated with GSE and dry-bonded was much higher than the non-cross-linked dry-bonded specimens and was even more than the wet-bonded specimens though the difference was not significant.

The results of the present study can be explained by the effects of cross-linking agents on the dentin and how they provide resistance to degradation of hybrid layer.

In a study done by Bedran-Russo et al. it was seen that the use of GSE resulted in a rapid and continuous increase in the modulus of elasticity of demineralized dentin. Increasing concentrations of GSE significantly increased the modulus of elasticity values, where the higher concentration of GSE resulted in the highest mean stiffness values.<sup>23</sup> Castellan et al. also performed a study on the mechanical interaction between proanthocyanidins and dentin matrix and found out that PCAs rich collagen cross-linkers like GSE increase mechanical properties and stability of dentin matrix.<sup>24</sup> Four mechanisms for interaction between PCAs and proteins have been proposed including covalent interactions,<sup>25</sup> ionic interactions,<sup>26</sup> hydrogen bonding interactions or hydrophobic interactions<sup>27</sup> that lead to increase in the elasticity of demineralized dentin. Along with elasticity, cross-linking agents also increased the ultimate tensile strength of demineralized dentin when treated with a PCA based agent.<sup>28</sup> Thus we can conclude that GSE as a cross-linking agent improves the mechanical strength of the demineralized dentin.

Moreover, pretreatment of acid-etched dentin matrices with crosslinking agents has also been seen to inactivate the endogenous proteases of dentin.<sup>29</sup> Host-derived proteases such as metalloproteinases (MMPs) and cysteine cathepsins play an important role in collagen fibrils degradation via peptide hydrolysis and thus destroy the hybrid layer. Cross-linking agents cross-link peptide chains in MMPs, thereby inactivating the enzyme by lowering the molecular mobility of the catalytic sites in these enzymes.<sup>29</sup> In a study done by Schaffer et al. inactivation of matrix-bound matrix metalloproteinases by cross-linking agents in acid-etched dentin was evaluated

and it was seen that along with inactivating the proteases, Cross-linking agents stiffen collagen polypeptides so that the proteolytic enzymes cannot unwind them and thus again prevent hydrolysis of the hybrid layer.<sup>30</sup>

In this study, when cross-linked dentin was dry-bonded, the bond strength of the specimens was as good as the wet-bonded specimens. This is because of the increased stiffness of the demineralised dentin due to cross-linking agent. We know that there is an inverse relationship between shrinkage and stiffness of demineralized dentin.<sup>31</sup> That is, as stiffness increases, shrinkage decreases. This allows the individual collagen fibrils to be separated from each other by air without getting collapsed.

Additionally, lack of interfibrillar spaces in the collapsed dentinal tubules results in loose resin tags that are not anchored to the surrounding intertubular dentin. The result is a micro tensile bond strength( $\mu$ TBS) of about 10 MPa that is insufficient to oppose the forces of polymerization contraction.<sup>32</sup> But in an SEM study done by Pashley et al. when acid-etched dentin was cross-linked with 5% GSE for 60s and then air-dried, the collagen fibrils did not collapse upon themselves and fuse together, as was seen in the non-cross-linked demineralized matrices when they were air-dried. Thus maintaining sufficient interfibrillar spaces to achieve  $\mu$ TBS of 55Mpa.<sup>33</sup> Similar results were obtained in this study where the shear bond strength was highest for the group which was cross-linked and dry-bonded.

In this study, the group pretreated with 5%GSE in ethanol gave the highest shear bond strength explaining the dehydrating effect of ethanol on the residual water. "Ethanol wet-bonding" was proposed by Tay et al. in 2007. In ethanol wet bonding, ethanol chemically dehydrates the acid etched dentin thus removing the residual water from the hybrid layer.<sup>12</sup> By getting rid of the residual water in dentin bonding, one reduces the risk

of hydrolyses of the dentin matrix by endogenous MMPs and cathepsins.

Moreover, in water-wet bonding, hydrophilic monomers are used to enhance their wetting properties and to avoid phase changes when hydrophobic dimethacrylates are added to water.<sup>34</sup> But it has been seen that adhesives containing hydrophilic resins exhibit high water affinity<sup>35</sup>, resulting in rapid deterioration of their mechanical properties.<sup>36</sup> In ethanol wet bonding, we can use hydrophobic monomers, yielding stronger and durable bonds. Microscopically ethanol wet bonding protocol has been seen to maintain interfibrillar width and hybrid layer thickness for resin infiltration and retention.<sup>37</sup> Therefore, in this study also, GSE mixed in ethanol gave the highest bond strength. The results are in accordance with a study done by Sadek FT et al. where coaxing hydrophobic resins into acid-etched dentin using ethanol-wet bonding gave one-year stability to resin-dentin bonds.<sup>38</sup>

### Conclusion

The results of this study demonstrate that acid-etched dentin cross-linked by 5% GSE in ethanol followed by air drying, does not lower bond strengths below wet-bonding levels of non-cross-linked dentin. Therefore, the study proves that even with dry bonding, we can get water-free, hydrophobic resin monomers filled hybrid layer that provides strong resin-dentin bonds and increase the durability of the bonds by eliminating the harmful effects of residual water in wet bonding. The results of this study need to be confirmed under in vivo conditions. Future long-term experiments on nanoleakage and bond strength over time should be done to test the results of this study to determine if clinicians can achieve more durable, resin-dentin bonds under in vivo conditions.

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