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Cross-linked dry bonding: A new etch-and-rinse technique

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ABSTRACT

Objective. To determine if acid-etched, cross-linked dentin can be dehydrated without lowering bond strength below that of cross-linked wet-bonded dentin *in vitro*.

Methods. Using extracted human third molars, control acid-etched dentin was bonded with Single Bond Plus, using either the wet- or dry-bonding technique. Experimental acid-etched dentin was treated with 5 mass% grape seed extract (GSE) in different solvents for 1 min before undergoing wet *vs* dry resin-dentin bonding with Single Bond Plus. Completely demineralized dentin beams were treated with 5% GSE for 0, 1 or 10 min, before measuring stiffness by 3-point flexure. Other completely demineralized beams were treated similarly and then incubated in buffer for 1 week to measure the collagen solubilization by endogenous dentin proteases.

Results. 24 h microtensile bond strengths (μTBS) in wet and dry controls were 53.5 ± 3.6 and 9.4 ± 1.8 MPa, respectively ($p < 0.05$). 5% GSE in water gave μTBS of 53.7 ± 3.4 and 39.1 ± 9.7 MPa ($p < 0.05$), respectively, while 5% GSE in ethanol gave μTBS of 51.2 ± 2.3 and 35.3 ± 2.0 MPa ($p < 0.05$). 5% GSE in 5% EtOH/95% water gave wet and dry μTBS of 53.0 ± 2.3 and 55.7 ± 5.1 MPa ($p > 0.05$). Cross-linking demineralized dentin with 5% GSE increased stiffness of dentin and decreased collagen degradation ($p < 0.05$).

Significance. 5% GSE pretreatment of acid-etched dentin for 1 min permits the dentin to be completely air-dried without lowering bond strength.

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1. Introduction

Water is one of the strongest hydrogen bonding solvents known, and has a Hoy's solubility parameter for hydrogen bonding cohesive forces (δ_h) of 40.4 $(J/cm^2)^{1/2}$ [1]. The intrinsic tendency of collagen peptides to form interpeptide H-bonds with each other in the absence of water is 14.8 $(J/cm^2)^{1/2}$ [2]. Such interpeptide hydrogen bonding cannot occur in the presence of water. That is, water molecules cluster around carbonyl oxygens and amide hydrogens in peptide bonds, which prevent direct hydrogen bonding between neighboring collagen peptides. The stiffness of demineralized dentin matrices is inversely related to the solubility parameter for hydrogen bonding cohesive forces of polar solvents [3,4]. Water is not only a solvent, but participates in many protein-water-coupled phenomena [5].

During cavity preparations, dentists expose mineralized tooth dentin that has a modulus of elasticity of 20,000 MPa [6]. To create microporosities in that dentin for resin-infiltration, they strip away the apatite crystallites in the mineralized matrix by acid-etching dentin, which solubilizes those crystallites to a depth of 10 μm [7]. After water rinsing to extract the residual acid and solubilized minerals, the exposed demineralized collagen fibrils have a modulus of elasticity of only 3–5 MPa [2]. As long as these collagen fibrils are suspended in water, they are very pliable. However, if that water is removed by evaporation or dehydrating solvents, the compliant collagen fibrils rapidly form interpeptide hydrogen bonds with their nearest neighbors. When this occurs, the 50–100 nm diameter collagen fibrils hydrogen bond to each other to form an impermeable membrane-like structure that prevents the permeation of solvated adhesive monomers around collagen fibrils [2,8]. This results in resin-dentin bond values of only 10 MPa. To avoid drying-induced shrinkage, and to create higher resin-dentin bond strengths, Kanca developed what is called the "wet-bonding technique" [9–11], where demineralized dentin is allowed to float in 70% water [7] during the monomer infiltration phase of dentin bonding. That bonding technique leaves far too much residual water in resin-dentin bonds [12,13], and provides hydrolytic fuel for the endogenous proteases of dentin matrices which slowly hydrolyze collagen fibrils in resin-bonded dentin, resulting in poor durability of resin-dentin bonds [7]. The goal of resin infiltration during dentin bonding is to replace all of the 70 vol% rinse water with 70 vol% adhesive monomers [7]. However, dimethacrylates such as triethylene glycol dimethacrylate are almost insoluble in water. They undergo phase changes from monomers in solution, to monomers in resin globules suspended in water [14–16]. Because these resin globules are too large to permeate through the 20 nm wide interfibrillar spaces, this results in significant amounts of collagen fibrils in hybrid layers being surrounded by water instead of polymerized resin [12]. To prevent phase changes, most manufacturers have added 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, monomethacrylates cannot produce strong cross-linked polymers. Rather, HEMA-rich polymers form elastomers that are not cross-linked. They are weak polymers which attract water to themselves that plasticizes their mechanical properties [17].

The authors propose to eliminate these problems by making the following modifications to the "wet-bonding technique". After rinsing away the unreacted acid and solubilized minerals, collagen fibrils suspended in water would be cross-linked by grape seed extract (GSE) [18–21] for 60 s. This agent is meant to be illustrative of cross-linking agents in general (i.e. carbodiimide, glutaraldehyde, etc.) [22,23]. The excess, unreacted cross-linker would then be rinsed away with water and the stiffened collagen fibril matrix air dried. There is an inverse relationship between shrinkage and stiffness of demineralized dentin [24,25]. That is, as stiffness increases, shrinkage decreases, allowing the individual collagen fibrils to be separated from each other by air.

The other problem is how to remove excess water. The vapor pressure of pure rinse water is much higher than it is after adding water-soluble adhesive monomers, which lower the vapor pressure of water (Raoult's Law) [26,27]. By evaporating the rinse water before adding primers or adhesives, it is possible to remove nearly all the rinse water added to dentin within 30 s using a strong, continuous air blast. In the absence of water, adhesive formulations free of HEMA and made entirely of dimethacrylates can be added to dry acid-etched, cross-linked dentin matrix [28]. The end result should be a hybrid layer free of residual water and filled with dimethacrylates that absorb little water [24]. Tay et al. [28] reported that ethanol-solubilized BisGMA could infiltrate ethanol-rinsed, acid-etched dentin using a new bonding technique called "ethanol wet-bonding" [2,29–31]. That bonding technique removed residual water by chemical dehydration with ethanol, an excellent solvent for dimethacrylates.

The purpose of the present work was to test three null hypotheses: (1) that there is no difference in the 24 h microtensile bond strengths (μTBS) of acid-etched dentin bonded to non-cross-linked wet *vs* dry specimens; (2) that there is no difference in the 24 h μTBS of acid-etched dentin bonded to GSE cross-linked dry *vs* GSE cross-linked wet specimens; (3) that there is no difference in the 24 h μTBS of acid-etched dentin bonded to non-crosslinked wet-bonded *vs* GSE cross-linked wet-bonded dentin.

2. Materials and methods

2.1. *Teeth used for resin-dentin bonding*

Thirty-two un-erupted human third molars were obtained from young (18–22 year old) patients in the Oral Surgery Clinics of The Dental College of Georgia at Augusta University with signed informed consent. They were stored in water containing 0.02% sodium azide as an antimicrobial, at 4 °C for less than 1 month before use.

2.2. *Cross-linking agent*

Proanthocyanidin was a gift from Dr. A. Bedran-Russo, who purchased it as Mega Natura-BP, from Polyphenolics, Madera, CA, USA. It was extracted from Vitis vinefera grapes and has been reported to contain 79.6 mass% total polyphenols [32]. It

was dissolved in water, ethanol or 5% ethanol/95% water at 5 mass%.

2.3. Bonding procedures

Occlusal enamel and superficial dentin were removed from the 32 extracted teeth using a Buehler diamond blade saw (Buehler Ltd., Lake Bluff, IL, USA) with copious water cooling. Then, the flat exposed mid-coronal dentin was sanded with wet 180-grit silicon carbide paper to create a standard smear layer [33,34]. The flat occlusal dentin surface of all teeth were acid-etched for 15 s with 37% phosphoric acid gel (3M ESPE, St. Paul, MN, USA). All etched teeth were rinsed with water for 60 s to remove unreacted acid and to extract solubilized mineral. In experimental specimens, the acid-etched dentin surface was treated for 60 s with one of the following GSE experimental cross-linking primers: 5 mass% GSE in water (pH 3.26), 5 mass% GSE in ethanol (pH 4.17), or 5 mass% GSE in 5% ethanol/95% water (pH 3.48). Cross-linking was then terminated by rinsing dentin surface with air-water spray for 10 s.

Specimens in the wet-bonded control group were not pretreated with GSE, and bonded using the wet-bonding technique. They were left visibly moist when bonding with Single Bond Plus (3M ESPE). Bonding was accomplished by application of two separate layers of solvated adhesive, followed by evaporation of the solvent for 5 s and light-curing for 40 s at 600 mW/cm^2 using an Optilux 500 halogen light (Demetron/Kerr, Danbury, CT, USA). Creation of resin composite build-ups was made using three 1.5 mm increments of Z100 resin composite (3M ESPE) that were individually light-cured for 20 s each.

Specimens in the dry bonded control group were not pretreated with GSE and had their wet dentin surfaces completely dried for 30 s with a continuous air blast at a distance of 10 cm. They were then bonded with Single Bond Plus to dry dentin.

Specimens in the cross-linked wet bonded group were treated with various GSE primers for 60 s, and were then rinsed for 10 s with the appropriate solvent (water, ethanol, or 5% ethanol/95% water). They were then lightly blotted with a Kimwipe tissue (Fisher Scientific, Pittsburgh, PA, USA) moistened with the same solvent and immediately bonded as previously described.

Specimens in the cross-linked dry bonding group were treated with various GSE primers for 60 s, 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 30 s. They were then immediately dry bonded with Single Bond Plus and built up with resin composite as described above.

The resin-bonded teeth were immersed in labeled, separate containers in 37 °C water for 24 h. Then, using an Isomet saw with water cooling, the curved peripheries of each bonded tooth were cut away to yield a square bonded crown. The resulting "squared" crown was cut into 0.7 mm thick slabs. Each slab was, in turn, cut into 0.7 mm thick "sticks". The μTBS of each stick was measured using Geraldeli testing jigs [35]. The tensile force at failure was recorded and divided by the cross-sectioned area of each stick, and expressed in MPa.

2.4. Creation of dentin beams for 3-point flexure and hydroxyproline release

One 0.5 mm thick dentin disk was obtained from each tooth using a Buehler diamond blade saw (Buehler Ltd.). Sixty dentin beams were cut from these disks that were 3 mm wide × 6 mm long, using the same saw. These beams were then completely demineralized in 10% phosphoric acid at 4 °C by tumbling in sealed containers for 18 h. Complete demineralization was confirmed by measuring the modulus of elasticity of beams in water. A modulus of elasticity of 5 MPa was considered as completely demineralized [36].

2.5. Measurements of stiffness of completely demineralized dentin

Due to the excellent μTBS results obtained from using 5 mass% GSE in 5% ethanol/95% water, we decided to use this solvent alone for the remaining experimental procedures.

Thirty demineralized dentin beams were used to measure stiffness by 3-point flexure. The initial elastic modulus of each beam was determined by means of a testing machine (Vitrodyne 1000, Liveco Inc., Burlington, VA, USA) with a 1000 g load cell, at a crosshead speed of 1 mm/min. Load-displacement curves were converted to stress–strain curves, and modulus of elasticity (E) was calculated at the steepest, most linear portion of the curve, using the formula $E = mL^3/4bd^3$, where m = slope (N/mm); L = support span (mm); d = thickness of beam (mm); b = width of beam (mm). After initial baseline testing in water, beams (10/group) were placed into 5% GSE in 5% ethanol/95% water for 1 min or 10 min. Immediately following incubation stiffening, the beams were rinsed in water and re-tested under the same parameters, and the new stiffness was measured. Each beam served as its own control.

2.6. Measurement of collagen solubilization by endogenous dentin proteases

Previous work from our laboratory showed that when completely demineralized dentin beams were incubated in buffer at 37 °C, they lost dry mass and stiffness [31]. This loss of dry mass was associated with solubilization of hydroxyproline-containing collagen peptides. This hydrolytic activity is due to the presence of endogenous proteases in dentin matrices, including MMPs -2, -8, and -9 and cathepsin K [7,37]. When these demineralized matrices were treated by cross-linking agents such as carbodiimide or glutaraldehyde, the loss of dry mass was significantly reduced [22,23,38].

Thirty 3 mm × 0.5 mm × 6 mm dentin beams were prepared from mid-coronal dentin as described above. After rinsing in water, the demineralized beams (10/group) were dipped in water for 10 min or in 5 wt% GSE in 5% ethanol/95% water for 1 min or 10 min, rinsed briefly and then dropped into 0.5 ml of 0.05 M HEPES buffer in sealed containers that were incubated at 37 °C for 1 week with shaking at 15 cycles/min. The HEPES buffer (pH 7.4) also contained 2.5 mM $CaCl_2{\cdot}2H_2O$ and 0.02 mM $ZnCl_2$ (both from Sigma–Aldrich, St. Louis, MO, USA). At the end of one week, 100 μL of incubation media was mixed with an equal volume of 12 N HCl to create 6 N HCl, which was used to hydrolyze the soluble collagen fragments into their

constituent amino acids in sealed glass ampoules at 118 °C for 16 h. After opening vials, the HCl was allowed to evaporate in a vacuum dessicator, the bottom of which was covered by NaOH pellets to neutralize the HCl. The dry residue was then analyzed for hydroxyproline using a colorimetric method [39].

2.7. Scanning electron microscopy

Control and experimental dentin specimens (3/group) were acid-etched with 37% phosphoric acid gel for 15 s, then rinsed with water for 15 s. The control specimens were then treated with water for 1 min. The experimental teeth were treated with 5 mass% GSE in water, ethanol or mixtures for 1 min. All surfaces were rinsed with water for 15 s and then air-dried for 30 s using continuous, full strength air for 30 s, 10 cm from the dentin surface. All specimens except those that were dry-bonded were critical-point dried (Samdri-790, Hummer Sputtering System, Anatech Corp., San Diego, CA, USA) prior to being coated with gold/palladium and examined in an SEM (Model XL-30 FEG, Philips Corp., Hillsboro, OR, USA) at 10 keV.

2.8. Statistics

The μTBS obtained from beams derived from each of the 4 teeth in each group (8 groups) were pooled together to obtain the mean bond strength value. Each tooth was treated as a statistical unit. The bond strength data were analyzed via two-way ANOVA (SigmaPlot 13, Systat Software Inc., San Jose, CA, USA) using cross-linking as one factor and bonding type (wet *vs* dry) as the second factor. There was a significant interaction between cross-linking and bond type ($p < 0.001$). Thus, the data were re-analyzed by the least squares means test. Least squares means are the expected value of group or subgroup means that one expects for a balanced design involving the group variable with all covariates at their mean value.

The matrix stiffness and collagen solubilization data were logarithmically transformed to obtain normal distribution and equality of variance. They were then analyzed using separate, one-way ANOVAs and Holm-Sidak multiple comparison tests (SigmaPlot 13). Statistical significance was set in advance at the 0.05 level.

3. Results

3.1. Microtensile bond strengths

When moist acid-etched, non-cross-linked dentin was treated with Single Bond Plus (wet-bonded control), the mean μTBS was significantly higher than the non-crosslinked dry-bonded control group ($p < 0.05$, Table 1). Cross-linking moist acid-etched dentin for 60 s with 5% GSE in all three solvents gave similar bond strengths that were not statistically different from the wet-bonded control group ($p > 0.05$, Table 1). Pretreating dentin with 5% GSE in water or ethanol and then air-drying for 30 s, produced μTBS values that were significantly lower ($p < 0.05$, Table 1) when compared to their wet-bonded values, although the μTBS values were still significantly higher than the non-cross-linked dry control specimens. When acid-etched dentin was cross-linked with 5% GSE in 5% ethanol/95% water and then air dried, there was no significant difference between its μTBS values and those values derived from wet-bonding ($p > 0.05$, Table 1).

Table 1 – Comparison of wet *vs* dry bond strengths of dentin pretreated with different versions of GSE solvents and dentin bonded without GSE pretreatment (control).

Groups	Wet bonding†	Dry bonding†
Control	53.5 ± 3.6[a]	9.4 ± 1.8[b]
5% GSE in water	53.7 ± 3.4[a]	39.1 ± 9.7[c]
5% GSE in 100% ethanol	51.2 ± 2.3[a]	35.3 ± 2.0[c]
5% GSE in 5% ethanol/95% water	53.0 ± 2.3[a]	55.7 ± 5.1[a]

† Values are mean ± standard deviations, $n = 4$ teeth. Means identified by different superscripts letters are statistically different (Holm-Sidak pairwise comparisons, $p < 0.05$).

3.2. Stiffness of completely demineralized dentin beams

After immersion in 5% GSE in 5% ethanol/95% water for 1 min, the mean stiffness of demineralized dentin beams increased significantly when compared to the control value (Fig. 1). Increasing the incubation time to 10 min produced stiffness values that were higher than the 1 min beams ($p < 0.05$, Fig. 1).

3.3. Collagen solubilization by endogenous dentin proteases

Control beams incubated in HEPES buffer for 1 week released high levels of hydroxyproline-containing peptides into the incubation medium (Fig. 2). Beams pretreated with 5% GSE in 5% ethanol/95% water for 1 or 10 min released significantly lower hydroxyproline than the control values ($p < 0.05$). One minute of GSE incubation reduced collagen solubilization by

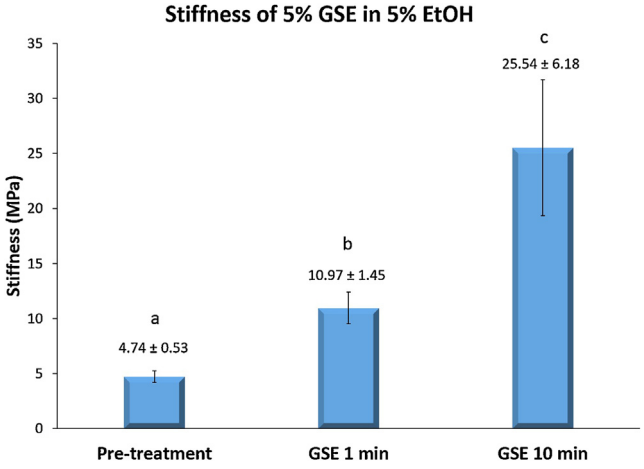

Fig. 1 – Modulus of elasticity of completely demineralized dentin beams subjected to 3-point flexure in water (control), and after 1 or 10 min immersion in 5 mass% grape seed extract. Data are presented in means ± standard deviations ($n = 10$). Groups identified by different lower case letters are significantly different (Holm-Sidak pairwise comparisons, $p < 0.05$).

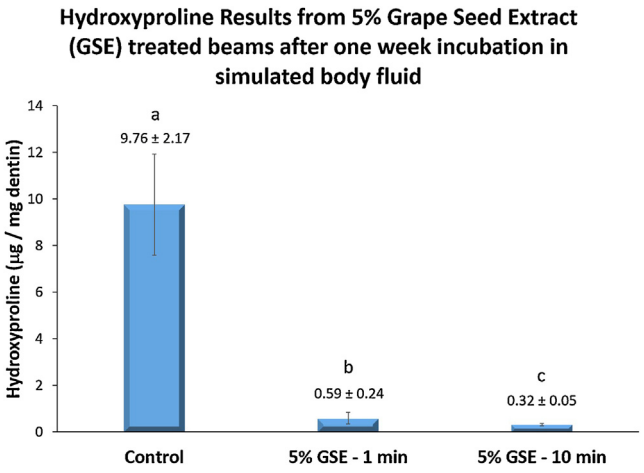

Fig. 2 – Release of hydroxyproline-containing soluble peptides from demineralized dentin (control) beams dipped in water for 10 min *vs* beams dipped into 5 mass% GSE dissolved in 5% ethanol/95% water for 1 or 10 min, rinsed and then incubated in 0.05 M HEPES buffer (pH 7.4) for 1 week at 37 °C with shaking. Heights of bars are the mean values of 10 beams per group. Brackets indicate ± 1 SD. Groups identified by different lowercase letters are significantly different at $p < 0.05$.

94%, while the reduction was 97% after 10 min (Fig. 2). These values were significantly different from each other.

3.4. *SEM of control vs GSE-treated dentin*

Fig. 3 shows the image of a dentin surface that was acid-etched with 37% phosphoric acid for 15 s, rinsed with water, and then critical-point dried to prevent collapse and fusion of collagen fibrils. The insert shows a higher magnification view of the acid-etched dentin surface that permits visualization of individual collagen fibrils separated from each other by interfibrillar spaces. These spaces serve as diffusion channels for inward adhesive monomer infiltration. At this magnification, the circumferential orientation of individual collagen fibrils lining the walls of the tubules can be clearly seen. This serves as a control for Fig. 4 which was treated similarly, but was allowed to be air dried for 30 s.

Fig. 4 is a representative SEM image of dentin that was acid-etched with 37% phosphoric acid for 15 s, rinsed with water and then air-dried with a continuous maximum air blast for 30 s at a distance of 10 cm. The intertubular dentin appeared to be smooth and amorphous. The tubules orifices have a "rolled-edge" appearance, unlike those seen in Fig. 3 where the orifices had sharp edges. No individual collagen fibrils can be seen between tubules. The "disappearance" of collagen fibrils was due to their fusion with each other by direct hydrogen bonding [2,7]. The intertubular dentin contains aggregates of fine granular material, representing silica that is added to the 37% phosphoric acid liquid to control the rheology of the gel etchant.

Fig. 5 is an image of acid-etched dentin that was treated with 5 mass% grape seed extract dissolved in 5% ethanol/95% water for 60 s and then rinsed with water for 10 s before 30 s of air drying. The texture of the intertubular dentin is not smooth and amorphous, but is rough. The circumferentially oriented collagen fibrils lining the tubules are well-separated, revealing interfibrillar spaces.

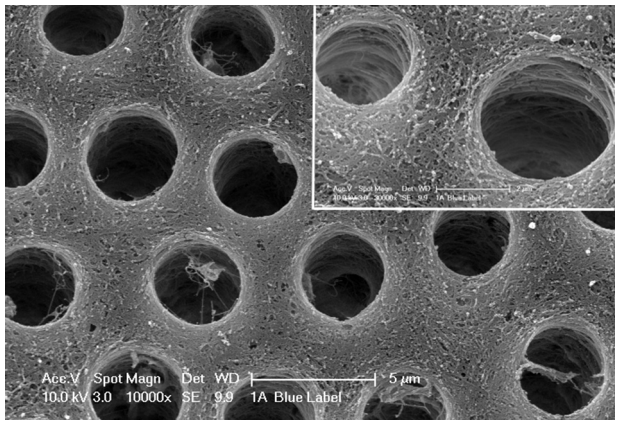

Fig. 3 – Scanning electron microscopy images of a mid-coronal crown dentin surface that was acid-etched with 37% phosphoric acid for 15 s and then rinsed with water for 10 s before being processed by critical-point drying (10,000×). Note the lack of peritubular dentin and the presence of individual collagen fibrils in the intertubular dentin and individual circumferential collagen fibrils within the dentinal tubules. INSERT. A higher magnification view of the same specimen (30,000×). Interfibrillar spaces could be identified between both the collagen fibrils in the intertubular dentin, and within the circumferential collagen fibrils with the lining of the tubules.

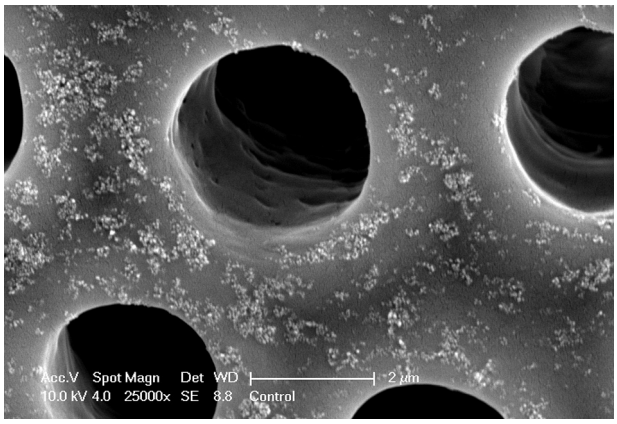

Fig. 4 – Scanning electron microscopy image of a mid-coronal crown dentin that was acid-etched for 15 s with 37% phosphoric acid, rinsed with water for 10 s and then air-dried for 30 s with continuous air blast at a distance of 10 cm (25,000×). Fine granular materials present on the surface of the collapsed intertubular dentin are silica gel used by 3 M to gel their phosphoric acid etchant. No individual collagen fibrils could be identified on the surface of the intertubular dentin or within the walls of the dentinal tubules because they have fused with each other by direct hydrogen bonding [7].

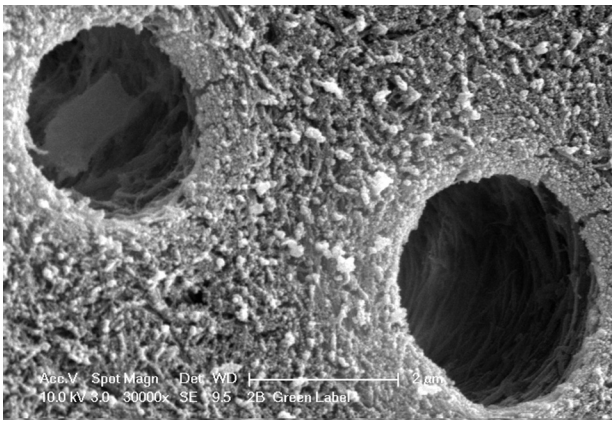

Fig. 5 – Scanning electron microscopy image of a mid-coronal crown dentin that was acid-etched for 15 s with 37% phosphoric acid and rinsed with water for 10 s. The acid-etched dentin surface was then treated with 5 mass% grape seed extract dissolved in 5% ethanol/95% water for 60 s, followed by rinsing for 10 s with water and air drying for 30 s with air blast. (30,000×). The intertubular dentin has a rough surface texture in which individual collagen fibrils can be recognized between the tubules and within tubules. Unlike the control in Fig. 4, the collagen fibrils in this figure did not collapse or fuse with each other.

4. Discussion

Because the μTBS value of non-cross-linked wet-bonded dentin was 53.5 ± 3.6 MPa, while that of non-cross-linked dry-bonded dentin was only 9.4 ± 1.8 MPa ($p < 0.05$), the results require rejection of the first null hypothesis. We speculate that non-cross-linked, dry-bonded specimens that were devoid of interfibrillar spaces did not permit resin-infiltration through the collapsed, air-dried surface [7]. We speculate that the low bond strengths were due largely to the presence of unhybridized resin tags in the tubules that are known to only contribute about 10 MPa to total bond strength [40].

Microtensile bond strengths of control, non-cross-linked dentin bonded using the wet bonding technique and specimens pretreated with 5 mass% GSE in all solvents under wet conditions were over 50 MPa, while those made to GSE in 100% water or ethanol under dry conditions were less than 40 MPa (Table 1). Cross-linked specimens pretreated with GSE in 5% ethanol/95% water gave high bond strengths to dentin under both wet and dry conditions. These data require partial rejection of the second null hypothesis.

Because there was no significant difference between the μTBS values of control wet-bonded dentin (53.5 ± 3.6 MPa) and GSE-cross-linked wet-bonded dentin (51–53 MPa, Table 1), the third null hypothesis cannot be rejected.

In the present study, GSE was used to cross-link and stiffen acid-etched dentin. Grape seed extract is a complex mixture of polyphenol monomers, dimers, trimers, tetramers, and polymers [41]. This cross-linking agent, while very effective, has a brown color that might prevent its use in esthetic dentistry. However, we also used 1.0 mass% GSE and obtained μTBS values that were as high as those produced by 5.0 mass% GSE, but had a much paler color than did the 5% solutions (data not shown). The use of GSE as a cross-linker is meant to be illustrative of results that may be achieved using cross-linking materials in general. The results obtained in the present study using GSE need to be confirmed in similar studies using glutaraldehyde or carbodiimide or other cross-linking agents. We speculate that by removing unbound water from acid-etched, cross-linked dentin matrices by 30 s of air-drying, more hydrophobic resin formulations free of HEMA and rich in dimethacrylates may be used to infiltrate the collagen matrix of dentin, without inducing the phase separations of dimethacrylates that are seen in water-wet bonding [14,15]. We demonstrated that BisGMA alone could be used to infiltrate acid-etched dentin using the ethanol-wet bonding technique to remove residual water [28]. This should also be possible using the cross-linking, dry bonding technique.

Pretreatment of acid-etched dentin matrices with cross-linking agents is known to inactivate the endogenous proteases of dentin [23,37] that are hydrolases. Cross-linking also prevents collagenases from unwinding the collagen triple helix that is necessary for collagenases to cleave all three polypeptides in the tropocollagen molecule [5,42,43]. In the absence of water, the cross-linked collagen can be infiltrated with dimethacrylates such as TEGDMA and BisGMA that are known to absorb little water [17], thereby extending the durability of resin-dentin bonds, one of the goals of the NIDCR's current research emphases. As resin-dentin formulations become more hydrophobic, they adsorb much less water than current hydrophilic formulations [17], reducing their tendency to become plasticized by water over time.

One possible explanation for GSE treatment preventing complete collapse of acid-etched dentin collagen fibrils is that the GSE becomes bound to collagen fibrils. Periodic binding may hold the fibrils apart, leaving sufficient room for resin infiltration. Alternatively, the collagen fibrils stiffness may have prevented the fibrils from complete collapse [22,23].

Clearly, non-cross-linked collagen fibrils collapsed completely when air-dried. There were no spaces between the collagen fibrils because, in the absence of water, they hydrogen bonded to each other [2]. Interfibrillar spaces between collagen fibrils serve as diffusion channels for resin monomer infiltration. Without interfibrillar spaces, resin infiltration of surface collagen fibrils will be incomplete. Additionally, lack of interfibrillar spaces in dentinal tubules results in loose resin tags that are not anchored to the surrounding intertubular dentin. The result is a μTBS of about 10 MPa that is insufficient to oppose the forces of polymerization contraction [44].

When acid-etched dentin was cross-linked with 5% GSE for 60 s prior to air-drying, 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 (compare Fig. 4 *vs* Fig. 5). Although 5% GSE in 5% ethanol/95% water cross-linked demineralized matrices did shrink some when air-dried, they left sufficient interfibrillar space to create μTBS values (55.7 ± 5.1 MPa) that were not different from wet bonded controls. Little is known regarding how much interfibrillar space is required for achieving optimal bond strength. Apparently, treatment of acid-etched dentin with 5% GSE in 5% ethanol/95 water prior to air-drying provides sufficient interfibrillar spaces to achieve μTBS of 55 MPa (Table 1).

An alternate explanation is that the polyphenols bind to dentin non-uniformly. When the polyphenol-coated collagen fibrils are air-dried, we speculate that the polyphenols hydrogen bond to their nearest neighbors, stiffening the matrix enough so that it does not collapse completely, but leaves sufficient interfibrillar spaces for resin infiltration.

The clear superiority of 5% ethanol/95% water as a GSE solvent for producing higher μTBS than either 5% GSE in 100% ethanol or in 100% water, requires more research. As GSE is a mixture of polyphenols, 5% ethanol/95% water may solubilize more of the larger oligomers [32] that contain additional hydroxyl groups that could provide more covalent and noncovalent bonds [32,45].

Three-point flexure of completely demineralized beams revealed that 10 min exposure to 5 mass% GSE extract increased the stiffness from 4.74 ± 0.53 MPa to 25.54 ± 6.18 MPa (Fig. 1). As the macrohybrid layer [2] specimens used in the 3-point flexure experiments were 0.5 mm thick beams, their thickness (500 μm) was 50 times thicker than acid-etched demineralized layers in resin-dentin bonds (10 μm thick). Thus, it is likely that the 10 μm demineralized matrix surfaces that are created clinically can become cross-linked much faster than the 500 μm-thick beams. Direct extrapolations between 10 μm demineralized layers to 0.5 mm-thick beams is fraught with risk, but it is clear that 1 min of 5% GSE in 5% ethanol/95% water treatment of an acid-etched layer probably stiffens the collagen fibrils far above their 4.74 MPa control values. That increase in stiffness may have prevented the complete collapse of collagen fibrils that was seen in the control SEM (Fig. 4) after air-drying.

The combination of cross-linking acid-etched dentin and dry-bonding needs to be tested with other cross-linking agents. The ability to remove water before it has been mixed with solvated adhesives permits more rapid water evaporation because the water vapor of pure water at 37 °C is 47 mm Hg. When solvated resin monomers are added, the vapor pressure of water can fall significantly [27,46], making it harder to evaporate solvents, including water, from dentin. Strong continuous air blasts for 30 s physically force much of the water from the matrix and then evaporate the residual water. By removing most of the free water, one reduces the risk of the phase changes that can occur when solvated dimethacrylates are mixed with water-saturated dentin. We speculate that in the absence of free water, there should be no portions of hybrid layers infiltrated with residual water instead of resin, and the endogenous hydrolases of the dentin matrix (i.e. MMPs and cathepsins) should be unable to cleave collagen. As residual water decreases, resin formulations containing more dimethacrylates can be utilized. The present study needs to be repeated using more hydrophobic resins [2,17,47]. Furthermore, these results need to be confirmed under *in vivo* conditions. The many advantages of cross-linked dry bonding need to be explored further and fully optimized before it can become a routine bonding practice.

5. Conclusion

This work is dedicated to the pioneering work of Professor Fusayama [48] who had the correct idea, but did not know that air-drying completely collapsed collagen fibrils, or how to avoid that collapse. The results of the present work demonstrate the proof-of-concept that acid-etched dentin stiffened by 60 s by 5% GSE in 5% ethanol/95% water cross-linking prior to air drying, does not restrict resin uptake enough to lower bond strengths below wet-bonding levels. We speculate that cross-linking, dry-bonding should permit the infiltration of water-free, dimethacrylate-rich, relatively hydrophobic resin monomer blends into such matrices, that should create strong resin-dentin bonds to dentin. Five percent GSE in 5% ethanol/95% water inactivated the endogenous proteases of dentin, and should increase the durability of the bonds. Future long-term experiments on nanoleakage and bond strength over time should test those hypotheses. The final test of such techniques is to determine if clinicians can achieve more durable, resin-dentin bonds under in vivo conditions.

Conflict of interest

None of the authors received payment from any manufacturers to support this work.

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