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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