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Comparative evaluation of acid etched, cross linked dry and wet bonding procedures against conventional bonding: an in vitro study

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Abstract

Objectives

To compare the micro tensile bond strength of acid-etched dentin bonded to grape seed extract cross linked dry and non-cross linked dry specimens.

To compare the micro tensile bond strength of acid-etched dentin bonded to grape seed extract cross linked wet and grape seed extract cross linked dry specimens.

Materials and Methods: The flat occlusal dentin surfaces of eighty teeth were acid etched for 15 seconds, rinsed for 30 seconds, and were divided into 4 groups of 20 each: Group A teeth specimens were gently blot dried. Group B teeth specimens were completely dried for 30 sec. Group C specimens were rehydrated with 5 mass% grape seed extract in 5%ethanol/95% water for 1 minute and lightly blot dried. Group D specimens were rehydrated with 5 mass% Grape seed extract in 5%ethanol/95%water for 1 minute and air dried for 30 seconds. 2 coats of dentin bonding agent were applied to surface treated tooth surfaces and individually light cured for 10 seconds each. Resin composite build-up was done on all the samples and individually light-cured for 20 secs. Specimens were sectioned horizontally to form beams of dimension 1mmx8mm. Specimens were stored in artificial saliva for 24 hours and evaluated for microtensile bond strength testing(μTBS).

Results: Pretreatment of acid etched dentin with 5% GSE showed a significant immediate increase in bond strength under both dry and wet conditions compared to conventional bonding techniques.

Conclusion: Within the limitations of this study, acid etched dentin stiffened by 5 mass GSE in 5%ethanol/95%water and air dried showed increased micro tensile bond strength compared to conventional dry bonding technique. Cross linked specimens pretreated with GSE showed similar results in both dry and wet conditions after 24 hours.

Keywords: Grape seed extract; micro tensile bond strength

Introduction

One of the greatest challenges in adhesive dentistry is to obtain a strong and stable bond between composite resin and dentin. Dry bonding and wet bonding have been suggested to achieve adequate bonding with etch and rinse adhesives.

In case of dry bonding, drying the cavity causes the acid-etched dentin to collapse. Such collapsed demineralized dentin loses the interfibrillar spaces that serve as channels for monomer infiltration [1]. This results in insufficient micro tensile bond strength to oppose forces of polymerization shrinkage finally leading to failure of restoration.

To avoid drying induced shrinkage, and to create higher resin-dentin bond strengths the concept of wet bonding was developed [2-4]. But this technique leaves too much of residual water in resin- dentin bonds [5,6]. Thus, bonding deteriorates over time by hydrolysis and enzymatic degradation by host-derived matrix metalloproteinases (MMPs) [7]. Hence wet bonding technique would not be able to achieve durable adhesion to dentin.

This study was done to address the problems associated with dry and wet bonding techniques i.e., low bond strength and poor durability of resin dentin bond, respectively. Application of grape seed extract as a cross linking agent has been suggested as a modification for conventional dry and wet bonding techniques.

Materials and Methods

Eighty extracted human premolars were collected after the patient's informed consent had been obtained and it was stored in 0.02% sodium azide solution (NICE). Occlusal enamel and superficial dentin were removed horizontally (Figure 1) (i.e., perpendicular to the long axis of the tooth) using a water-cooled diamond disc. Then, the flat exposed mid- coronal dentin was sanded with wet 180 grit silicon carbide paper to create smear layer.

Preparation of experimental solution: (Figure 2)

Grape seed extract was available as 150mg capsule (Healthy Origins, Mega Naturals BP, California). A 5% solution was prepared by dissolving the contents of the capsule in 3mL of ethanol (0.5ml ethanol and 9.5ml water)

Bonding procedures

The flat occlusal dentin surfaces of all teeth were acid etched for 15 seconds with 37% phosphoric acid gel (3M ESPE), rinsed for 30 seconds with distilled water.

After acid etching, teeth were divided into 4 groups:(Grouping based on surface treatment of teeth)

1. Wet bonding control/ Group A: 20 Nos. Teeth specimens were gently blot dried.
2. Dry bonding control/ Group B: 20 Nos. The wet dentin surfaces were completely dried for 30 sec with a continuous air blast.
3. Cross linked wet bonding/ Group C: 20 Nos. The acid etched dentin surfaces of Group C specimens were rehydrated with 5 mass% grape seed extract in

5%ethanol/95%water (Healthy Origins, Meganaturals BP) for 1 minute and lightly blot dried.

4. Cross linked dry bonding/ Group D: 20 Nos. The acid etched dentin surfaces of Group D specimens were rehydrated with 5 mass% grape seed extract in 5%ethanol/95%water (Healthy Origins, Meganaturals BP) for 1 minute and air dried for 30 seconds.

Two consecutive coats of dentin bonding agent (Adper™ Single Bond 2) were applied to surface treated tooth surfaces and individually light cured for 10 seconds. Resin composite (Z100 resin composite, 3M™ ESPE) build-up of 4mm was done on all the samples by incremental layering technique and individually light cured for 20 seconds using LED curing light (3M™ Elipar deep cure).

Micro tensile bond strength

Specimens were then sectioned horizontally measuring a total of 8mm height from the level of composite build up to cervical area (i.e., 4mm dentin and 4mm composite). From this section 1mmX 8mm beams were made and three to five such beams were made from each tooth. Specimens were stored in artificial saliva for 24 hours before subjecting to microtensile bond strength testing. Each beam was attached on either side of customized wooden jigs (Figure 3) using araldite glue and subjected to tensile load in Universal testing machine (Shimadzu) until failure. (Figure 4)

Results

Microtensile bond strength tests were done to evaluate the effect of 5 mass% grape seed extract in 5%ethanol / 95%water on dentin bond strength. The bond strength of all actual tested specimens was analyzed by ONE WAY ANOVA AND POST HOC TUKEY TEST, (Tables 1 and 2). Comparisons between the bond strength of control group and experimental groups was done after 24 hours.

Both the experimental groups (Table 1) responded positively to collagen crosslinking and anti-collagenolytic treatment with proanthocyanidin, resulting in a significant increase in dentin bond strengths.

Post Hoc Tukey test (Table 2) allows multiple comparisons within the groups. These analyses are usually concerned with finding patterns or relationships between subgroups of sampled populations. It helps for the intergroup comparison.

Microtensile bond strength

PostHoc Tukey tests comparing conventional dry bonding and cross-linked dry bonding groups shows a mean difference of 39.31 and is statistically significant with a p value of 0.0001. Comparing cross-linked dry and cross-linked wet bonding groups shows a mean difference of 2.16 and is not statistically significant with a p value of 0.131.

DISCUSSION

Adhesive dentistry has advanced over the last decade and a major part of this achievement is due to the improvements made in dentin bonding. However, despite various advancements in dentin bonding agents, contemporary adhesives still face issues related to poor dentin bond strength in etch and rinse as well as self-etch adhesives.

The bulk of the tooth is formed by dentin. Human dentin is approximately 70wt% inorganic material, 18wt% organic material, which is largely composed of collagen fibers type I, and 12wt% of water [10]. When etchant is applied to dentin it causes demineralization of superficial hydroxyapatite and removes the smear layer and smear plugs to expose the collagen fibrils that remain anchored to the underlying mineralized dentin and open the dentinal tubules.

During vigorous air desiccation of acid etched dentin, hydrogen bond formation occurs amongst the collagen fibrils resulting in their collapse. Thus, the interfibrillar spaces that are available as diffusion channels for resin

infiltration disappears. This results in extremely low resin-dentin bond values of only 10MPa [11]. Thus, collagen hydration is necessary.

Water provided by rinsing or that present in the subsequently applied hydrophilic primer prevents collapse of the collagen fibrils. The dentin bonding is primarily diffusion based which depends on the hybridization or infiltration of hydrophobic adhesive resin into the exposed collagen mesh of inherently wet dentin [13]. Adhesives are applied with the expectation that the resin monomer would completely penetrate the nano porosities of demineralized dentin matrix and dentinal tubules resulting in complete hybridization. However, the adhesive monomers are unable to fully impregnate the exposed collagen matrix, thereby leaving collagen fibrils partly or completely exposed at the bottom of hybrid layer. The lack of polymerized resin and presence of water leaves the collagen fibrils prone to hydrolytic breakdown contributing to destruction of hybrid layer and loss of dentin bond strength [14].

The hydrophilic nature of the adhesive resin increases due to presence of 2-hydroxyethylmethacrylate(HEMA), that absorbs more water contributing to the effect of plasticization thereby reducing the mechanical properties of the adhesive joint of the resin component [12]. Dimethacrylates such as triethylene glycol dimethacrylate (TEGDMA) experience phase changes from monomers in solution to monomers in resin globules suspended in water [8]. Since these resin globules are too large to permeate through the 20nm interfibrillar spaces, substantial quantities of collagen fibrils are surrounded by water instead of polymerized resin in hybrid layers [5].

Different approaches have been devised to prevent and control the hydrolytic breakdown of hybrid layer and the subsequent loss of bond strength. The most tested method is by using chlorhexidine which efficiently prevents hydrolysis of the collagen fibrils by matrix metalloproteinase (MMP-2, -8, -9) and cysteine cathepsin and the resultant hybrid layer degradation. However, chlorhexidine may leach out of the hybrid layers within 24 months [15]. Other techniques which aim at eliminating hydrolysis of collagen and resin components includes ethanol wet bonding and biomimetic remineralization. Yet another interesting solution is inactivation of endogenous proteases by using cross linking agents.

Cross linking agents includes glutaraldehyde, grape-seed extract, carbodiimide, low-dose riboflavin etc. In this study grape seed extract was used as the cross-linking agent to stiffen the acid etched dentin. Proanthocyanidin (PA) was available as a 150 mg capsule from Mega Natural-BP. It was extracted from Vitus vinifera seed which contains 90% polyphenols.

Polyphenols represents the third most abundant constituents in grapes which includes flavonoids and phenolic acids. Flavan-3-ol monomers include catechin, gallocatechin, epicatechin, and epigallocatechin, etc. Catechins condense into dimers and polymeric compounds otherwise called as condensed tannins. These condensed tannins also known as proanthocyanidins is the active content which is responsible for its cross-linking property with the collagen fibers [16]. Thus, in addition to their anti-bacterial, antioxidant, anti-inflammatory, vasodilatory properties proanthocyanidins possesses cross linking ability. Interaction of proanthocyanidin with collagen may be explained via four mechanisms- covalent interactions, ionic interactions, hydrogen bonding or hydrophobic interactions [17-19].

Grape seed extract and cocoa seed extract are ideal sources of proanthocyanidin. Grape seed extract has low toxicity and inhibits more than 90% of metalloproteinase MMP-2, MMP-8, and MMP-9 and approximately 75-90% of cysteine cathepsin B and K in dentin. Proanthocyanidin

increase type I collagen fibril cross linking resulting in improved mechanical properties such as modulus of elasticity and hardness [21].

The proanthocyanidin treatment on teeth could be in the form of application as a primer after acid etching procedure, or incorporated in self-etch primer, or incorporating PAs into dental adhesives, or as a PA-combined acid etchant in etch and rinse technique. In this study, grape seed extract as a cross linking primer was utilized as proanthocyanidin molecules can also replace water present in the extra fibrillar spaces thereby functioning as a primer [20].

Earlier literature on proanthocyanidin as a primer reported application times varying from 10 minutes to 1 hour which is a clinically unfeasible option. Liu Y et al., in their study to investigate clinically relevant time periods for proanthrocyanidin to exhibit dentin collagen cross links demonstrated through spectroscopic studies that proanthocyanidin can effectively cross link collagen even after a 10 second application time [22]. In a study by Liu R et al., proanthocyanidin cross linkers were used in clinically applicable durations (60 seconds/120 seconds) increased the cross-linking degree and bond strength of acid etched dentin matrices [23]. In this study pretreatment of acid etched dentin surfaces with grape seed extract was done for 60 seconds to make this study clinically relevant.

When cross linkers such as proanthocyanidin are applied over the acid etched dentin it induces a stiffening effect which reduce collagen collapse following demineralization and dentin over drying, and henceforth, enable hydrophobic adhesives to enter interfibrillar spaces [9]. In this study, using grape seed extract as a cross linking agent under dry bonding conditions significant improvement in immediate microtensile bond strength was obtained compared to tooth specimens treated by conventional dry bonding technique.

Air dried acid etched, cross-linked dentin matrices can remove unbound water, thus HEMA free hydrophobic resin formulations can be applied as adhesives. Hence, dimethacrylates can easily penetrate through the interfibillar spaces without phase separations [11].

The proanthocyanidin content is critical to determine the cross-linking effect. Castellan et al., analyzed the elastic response of dentin matrix with natural agents rich in proanthocyanidin (grape seed, cocoa, cranberry, açaí berry and cinnamon) for different exposure times and storage for 12months. Higher stiffness values were obtained with grape seed extract and cocoa seed extract due to their proanthocyanidin contents, 95% and 45% respectively [24]. Bedran-Russo et al., in their study to examine the effect of glutaraldehyde and grape seed extract on the stiffness of demineralized dentin found that with increasing concentrations of grape seed extract there was a proportional increase in the modulus of elasticity [25].

The solvent used with grape seed extract also affects the proanthocyanidin- collagen interactions. The amount of hydrogen bonding is described by Hansen solubility parameter (δH) which is less with ethanol and acetone compared to distilled water. Therefore, if proanthocyanidin is mixed with ethanol or acetone more hydrogen bonding sites will be available for proanthocyanidin- collagen interactions [26]. Water if used as a vehicle for proanthocyanidin may combine with adhesives and prevent the permeation of the hydrophobic resin through the interfibrillar collagen spaces. Thus, it results in poor encapsulation of collagen fibrils by resin [8]. Hagerman and Klucher et al., reported ethanol as the solvent of choice for proanthocyanidin containing powders, as ethanol decrease the dielectric constant of the media thereby extending proanthocyanidin collagen

interactions [27]. Fang M et al., reported that proanthocyanidin pretreatment increased the microtensile bond strength of ethanol/water-based adhesive as compared to acetone / water -based system [28].

Zhou et al demonstrated higher microtensile bond strength when 5% ethanol / 95% water was used to mix grape seed extract compared to 100% ethanol or 100% water [11]. This may be because as GSE is a mixture of polyphenols, 5% ethanol/95% water may solubilize more larger oligomers containing additional hydroxyl groups of the GSE [29]. Thus, more hydroxyl groups are available for PA- collagen fibrils interactions.

Proanthocyanidin is a non-specific MMP inhibitor. Busenlehner and Armstrong reported activity of cross linkers against MMPs by bringing about conformational changes in enzyme structure [30]. Covalent cross links produced by external cross linkers inactivate the active sites of dentin proteases by two mechanisms. First, by decreasing the molecular mobility of active site and secondly by converting carboxy groups into amide groups [14].

In this study a comparative analysis between different bonding procedure were carried out. From the results obtained low micro tensile bond strength was observed in teeth specimens that were air dried completely after acid etching. But significant improvement in the microtensile bond strength was obtained when acid etched dentin surfaces were cross linked before complete air drying.

However, no significant difference between the microtensile bond strength of the two-cross linked specimens could be obtained under wet and dry conditions. This is in accordance with a previous study by Zhou et al., where similar results were obtained with 5% grape seed extract pretreatment of acid etched dentin which concludes that collagen cross linking allowed dentin to be completely air dried without lowering bond strength below wet bonding levels [11].

In the present study only caries free sound teeth were used. However, this is not the ideal substrate since we frequently work with carious dentin clinically. Even though significant improvement in immediate bond strength have been noted with proanthocyanidin pretreatment it is essential to evaluate the long-term behavior of such pretreated dentin matrices for bond durability. Further studies should also be undertaken to determine the interactions occurring between collagen and proanthocyanidin in situ and whether there is any leaching out of proanthocyanidin from collagen in the long run.

Conclusion

According to the findings and within the limitations of the study it was concluded that:

Acid etched dentin stiffened by 5 mass% grape seed extract in 5%ethanol / 95%water and air dried showed increased micro tensile bond strength compared to conventional dry bonding technique. Cross linked specimens pretreated with grape seed extract showed similar results in both dry and wet conditions after 24 hours.

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Legend Figures and Tables

Figure 1: Occlusally reduced specimens

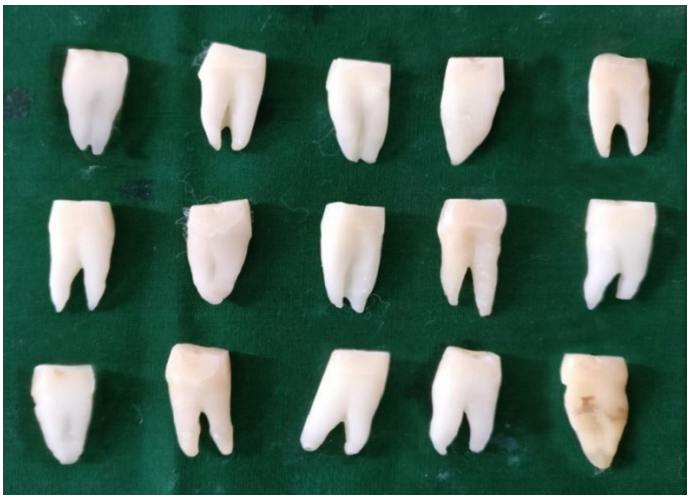

Figure 2: Grape Seed Extract

Figure 3: Tooth Specimen mounted on wooden jig

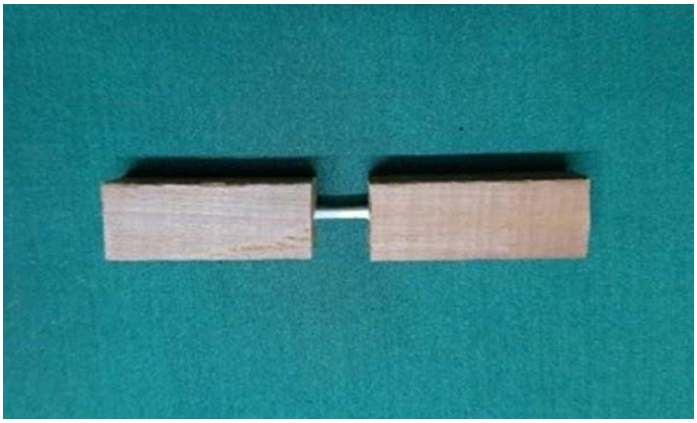

Figure 4: A fractured specimen

Table 1: Comparison of micro tensile strength:

Variable		A	B	C	D	F Value	P Value
Micro Tensile Bond Strength	Mean	45.371	12.194	49.348	51.509	491.306	0.0001*
	Sd	3.3	2.3	2.6	5.7		

P<0.05 is statistically significant (One Wat Anova test).

Table 2: Tukey's HSD Post-Hoc test to compare within the groups

Group(I)	Group(J)	Mean difference (I-J))	95% of confidence interval		P VALUE
			Lower	Upper	
A	B	-33.17	-36.2692	-30.0848	0.0001*
	C	3.97	0.8848	7.0692	0.0062*
	D	6.13	3.0458	9.2302	0.0001*
B	C	37.15	34.0618	40.2462	0.0001*
	D	39.31	36.2228	42.4072	0.0001*
C	D	2.16	-0.9312	5.2532	0.131

*P<0.05 is statistically significant (Turkey's Post Hoc test)