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Can 1% chlorhexidine diacetate and ethanol stabilize resin-dentin bonds?

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Abstract

Objectives—To examine the effects of the combined use of chlorhexidine and ethanol on the durability of resin-dentin bonds.

Methods—Forty-eight flat dentin surfaces were etched (32% phosphoric acid), rinsed (15 s) and kept wet until bonding procedures. Dentin surfaces were blot-dried with absorbent paper and re-wetted with water (Water, control), 1% chlorhexidine diacetate in water (CHD/Water), 100% ethanol (Ethanol), or 1% chlorhexidine diacetate in ethanol (CHD/Ethanol) solutions for 30 s. They were then bonded with All Bond 3 (AB3, Bisco) or Excite (EX, Ivoclar-Vivadent) using a smooth, continuous rubbing application (10 s), followed by 15 s gentle air stream to evaporate solvents. The adhesives were light-cured (20 s) and resin composite build-ups constructed for the microtensile method. Bonded beams were obtained and tested after 24-hours, 6-months and 15-months of water storage at 37°C. Storage water was changed every month. Effects of treatment and testing periods were analyzed (ANOVA, Holm-Sidak, $p < 0.05$) for each adhesive.

Results—There were no interactions between factors for both etch-and-rinse adhesives. AB3 was significantly affected only by storage ($p = 0.003$). Excite was significantly affected only by treatments ($p = 0.048$). AB3 treated either with ethanol or CHD/ethanol resulted in reduced bond strengths after 15 months. The use of CHD/ethanol resulted in higher bond strengths values for Excite.

Conclusions—Combined use of ethanol/1% chlorhexidine diacetate did not stabilize bond strengths after 15 months.

Keywords

ethanol-wet bonding; chlorhexidine; dentin; bonding; stability

Introduction

Resin-dentin bonds are currently accepted as being more complex than previously thought [1-3]. A lack of bond stability has been observed in several *in vitro* [4-7] and *in vivo* [8-10] studies for periods as short as 3 months [11]. The hydrophilicity of contemporary etch-and-rinse adhesives and subsequent hydrolysis [12, 13] in combination with host-derived enzymatic degradation of collagen fibrils [14-17] have been regarded as the two major causes of degradation of resin-dentin bonds over time.

Simplified etch-and-rinse adhesives incorporate hydrophilic monomers and solvents to properly bond to dentin, a naturally wet substrate. However, the use of increasing concentrations of hydrophilic resins raises concern that such adhesives have become too hydrophilic [18]. The incorporation of hydrophilic monomers results in increased water sorption that expedites hydrolysis and decreases mechanical properties [12, 19-21]. Bonding to wet dentin has also been shown to be challenging even with the use of hydrophilic adhesives. The surface moisture required for collagen expansion [22-24] may also cause phase separation of some etch-and-rinse adhesive systems, thus resulting in poor resin infiltration to the deepest regions of the demineralized dentin [25-27]. Conversely, air-drying dentin to eliminate water also results in poorly infiltrated hybrid layer [28, 29]. The exposed, uninfiltrated collagen fibrils are then susceptible to the enzymatic action of host metalloproteinases (MMPs) [15] that ultimately results in deterioration of the bond over time [23, 24, 30, 31].

Adhesive formulations for simplified etch-and-rinse systems incorporate either ethanol or acetone to solvate hydrophobic monomers. These solvents also function as water-chasers to displace entrapped water simultaneously to adhesive infiltration [32]. Anhydrous solvents play an important role in collagen matrix shrinkage, expansion, stiffness and overall infiltration [33-35]. The ethanol wet-bonding concept has been presented as an alternative technique to overcome problems associated with the collapse of the collagen matrix if water is removed from the surface [36, 37]. As ethanol has been shown to be able to expand and maintain collagen fibrils apart, it can be used to replace water, leaving demineralized dentin saturated with ethanol. This concept has been proved successful when used with experimental adhesive resins [38-40] or commercial etch-and-rinse adhesives [41, 42]. Ideally, protection and preservation of collagen should be achieved by complete infiltration of hydrophobic resins. This can be accomplished with the use of the ethanol wet-bonding concept [36, 37, 40].

Additionally, the incorporation of MMP inhibitors into the bonding procedure is desirable. *In vivo* and *in vitro* studies have shown that the application of aqueous solutions of 2% chlorhexidine digluconate plays an important role in preservation of resin-dentin bonds by inhibiting the collagenolytic activity of host-derived enzymes [14, 15, 17, 30, 31, 43]. Several studies have proposed chlorhexidine diacetate (CHD) as a potential bio-active antibacterial

agent to be incorporated to resin composites, glass ionomers, adhesives and provisional cements [44-48]. Chlorhexidine diacetate was selected in this study because it is available as a powder and is soluble in ethanol. It has been demonstrated that chlorhexidine digluconate concentrations in the range of 0.002 to 0.2% applied for shorter periods of time (15 to 30 s) are also capable to postpone the resin–dentin degradation of adhesive interfaces [49, 50]. Thus, the combined use of chlorhexidine and ethanol could represent a promising bonding technique. Although the effects of ethanol bonding and chlorhexidine on the durability of bond strengths to dentin have been extensively investigated in separate, the combination of both approaches in a clinically feasible protocol still requires further investigation.

This study investigated the hypothesis that the combined use of ethanol wet-bonding with 1% chlorhexidine diacetate in a short, and clinically feasible application time, would result in more stable resin-dentin bonds over time. The null hypothesis tested was that there is no effect of 1% chlorhexidine diacetate, ethanol or the combination of both on the stability of resin-dentin bond strength.

Materials and Methods

Tooth preparation

Forty-eight extracted human caries-free third molars stored in saline containing 0.1% thymol at 4°C for no longer than 6 months were used in this study. The study was approved by the Institutional Review Board of the university (# 164/07). A flat surface was prepared with a slow-speed Isomet saw (Isomet 1000 Precision Saw, Buehler Ltd., Lake Bluff, IL, USA) by transversally sectioning the crowns under water cooling to expose mid-coronal dentin. The dentin surface was polished (Ecomet 3000, Buehler Ltd., Lakebluff, IL, USA) with 320 and 600-grit SiC paper at 250 rpm to create a standard smear layer. The crown segments were randomly allocated to 8 groups of 6 teeth each. There were 4 solutions for dentin treatment (1% chlorhexidine diacetate in water, w/w) [CHD/W], 1% chlorhexidine diacetate in ethanol, w/w [CHD/E], distilled water [W, control] and 100% ethanol [E]); and 2 adhesive systems (Table 1), All Bond 3 (Bisco Inc.) and Excite (Ivoclar Vivadent), comprising 8 test groups. The adhesives were selected as representatives of commercial, water-free, ethanol-based, simplified etch-and-rinse systems. This was relevant to prevent confounding effect when using the ethanol-wet bonding approach.

Preparation of chlorhexidine diacetate solutions and bonding procedures

Chlorhexidine diacetate hydrate (Acros Organics, Fisher Scientific, Catalog number AC: 21498-0050) was used to prepare the experimental solutions. Solutions were prepared by gradually adding 1% by weight (Mettler Toledo, XP504 Delta Range) of chlorhexidine diacetate monohydrate to stirred 100% ethanol or water in a glass beaker. One single batch of the solutions was prepared, kept in the refrigerator and used for all bonding procedures where appropriate. No chlorhexidine diacetate precipitation was observed after the solutions were prepared or during the course of the experiment. The pH of the solutions was determined (Mettler Toledo, SevenMulti, pH mV/ORP, Schwerzenbach, Switzerland) as being 7.5 and 9.1 for water and ethanol solutions, respectively. They were not adjusted to a neutral pH.

As a standard procedure for all groups, tooth surfaces were acid-etched with 32% H₃PO₄ gel for 15 seconds (Uni-etch BAC 32%, Bisco Inc., Schaumburg, IL, USA), rinsed with water for 15 seconds and kept wet until bonded. The surface was blot-dried with tissue paper (Kimwipes, Kimtech Science) before further treatment according to groups. Dentin surfaces remained slightly moist, but no excess water was present. One of the 4 solutions was applied and kept in the surface for 30 seconds. The solutions were re-applied in the event of evaporation before 30 seconds, never allowing the ethanol-saturated dentin to evaporate to dryness. At the end of the 30 seconds, excess solution was blot dried with tissue paper. The respective adhesive was immediately applied with smooth rubbing action [51] for approximately 10 seconds, gently air-dried for 15 seconds. For All Bond 3, it was necessary perform a mixture of primers A and B before being applied. The third-step (adhesive resin layer) was omitted as permitted by the instructions. The rationale was to evaluate both ethanol-based adhesive systems under the same condition (as simplified etch-and-rinse adhesives). An additional, relatively hydrophobic layer could compromise the interpretation of the results because it is related to more stable bond strengths to dentin over time [5]. All groups were light-cured at 500mW/cm² (OptiLux 501, SDS KERR, Middleton, WI, USA) for 20 seconds. Immediately after bonding, the entire dentin surface received four layers of Aelite All Purpose Body resin composite (Bisco Inc., Schaumburg, IL, USA), (Table 1) to build an approximate 4 mm crown. Each 1 mm increment was light-cured for 40 seconds (OptiLux 501, SDS KERR, Middleton, WI, USA).

Preparation of specimens for microtensile test and storage

The bonded teeth were stored in water at 37°C for 24 hours, and then sectioned perpendicular to the adhesive-dentin interface using an Isomet diamond saw (Isomet 1000 Precision Saw, Buehler Ltd., Lake Bluff, IL, USA) to obtain rectangular beams of approximately 0.8 mm² cross-sectional area. One-third of the beams obtained from each tooth were randomly selected and tested immediately after sectioning, while the remaining two-thirds were kept in a clear vial containing neutral (pH = 7) distilled water at 37°C. Storage water was renewed monthly to expedite storage effects [52]. Preservatives and/or antimicrobial agents were not used in the storage water in this study.

Microtensile testing

Beams were tested immediately after sectioning as described above and then after 6 and 15 months of storage. Bonded beams were mounted on a microtensile testing jig with cyanoacrylate glue and pulled to failure at 1.0 mm/min in a Microtensile Tester machine (Bisco Inc., Schaumburg, IL, USA). The load (Kgf) at failure was divided by the cross-sectional area of the interface (mm²) measured with a digital caliper to the nearest 0.01 mm (Fisher Scientific, Chicago, IL, USA) to calculate the microtensile bond strength that was expressed in MPa. The bond failure modes were evaluated under 40× using light microscope (Olympus, Tokyo, Japan).

Fractures were classified as cohesive, adhesive or mixed. When it occurred exclusively in either dentin or composite, as cohesive in dentin (CD) or cohesive in composite (CC). As adhesive (A) when occurred at dentin/resin bonded interface, and mixed (M) when two modes of failures, adhesive and cohesive, occurred simultaneously.

Data treatment

Bond strength values were averaged per tooth for each experimental condition and adhesive. The means calculated from 6 teeth were then considered for further analysis ($n=6$). There were no pre-test failures of beams.

A two-way ANOVA design with the general linear model was used to examine the effects of four treatments, and three storage times, on microtensile bond strength per adhesive (SigmaPlot 11, Systat Software Inc.). Least-square means (LSM) analysis was used and the variances were given in standard error of the mean (SEM) [53]. *Post hoc* multiple comparison tests were performed with Holm-Sidak method. Significance level was pre-set to $\alpha = 5\%$.

Results

Bond strength

The bond strength values for AB3 were not affected by the cross-product interaction ($P = 0.480$), as well as, main factor surface treatments ($P=0.291$), but they were affected by the main factor storage time ($P=0.003$). Bond strengths dropped significantly from 24 hours to 15 months after water storage. For Excite, there was a statistically significant effect only for treatments ($P=0.042$), but not for storage period ($P= 0.644$) or interactions between factors ($P = 0.913$). When dentin was treated with Ethanol/CHD mix, Excite bond strengths were significantly higher than when treated with Water/CHD mix, but were not different from water or ethanol treatment alone at all storage periods (Table 2).

Failure mode analysis

The bond failure modes are summarized in Tables 3.1 and 3.2. Overall analysis showed 30% of cohesive failures in dentin for AB3 and 20.7% for Excite. Percentages of cohesive failures in dentin for 6 and 15-month were higher than for 24 hours groups for both adhesives evaluated. Ethanol treated groups, regardless of chlorhexidine treatment showed 29% of cohesive failures in dentin and 20% of adhesive failures. Mixed failures represented 45% and 32% of the total of failures for Excite and AB3, respectively.

Discussion

The bond strengths of the control groups of both adhesives were stable after 15 months of water storage. Therefore, a clear benefit from the use of the ethanol wet-bonding technique, with or without CHD was not evident. Ethanol saturation of dentin as originally described was achieved by using a series of ascending ethanol concentration, taking approximately 3-4 min, and which makes the technique unfeasible clinically. More recently, it has been demonstrated that a simplified ethanol-wet bonding technique (100% ethanol for 30 s) resulted in significant lower bond strength after 24 hours and six-month of aging when bonds were performed with experimental hydrophobic adhesives [41]. This current study demonstrated that a simplified ethanolwet bonding technique is feasible when using commercial etch-and-rinse adhesive systems in a clinically relevant time (i.e. 30 s). Bond strengths similar to the ones obtained in the conventional water wet-bonding technique were

obtained in ethanol wet-bonding technique for both simplified adhesives tested, and they all showed stability after a period of six-month water storage. Nishitani and co-authors [38] also demonstrated that moist ethanol bonding in a simplified version (20 seconds for water replacement) produced significantly higher bond strengths when compared with water moist surface for 5 experimental resins tested. However, the same study demonstrated significantly lower bond strengths for the most hydrophobic resin applied in ethanol moist surface. It appears that when highly hydrophobic resins are used for bonding, the original time-consuming water replacement with ascending ethanol concentrations is necessary for improved bond strengths. However, when less hydrophobic or commercial etch-and-rinse adhesives resins are used, the reduced-time ethanol-bonding technique (20 to 30 s water replacement) appears to be effective to produce high and stable resin-dentin bonds [38, 41]. While the ethanol wet-bonding concept has been presented as an alternative for bonding relatively hydrophobic resins, solvated or not, to dentin [38, 39, 53, 54], the present study added important information to the use of commercial etch-and-rinse adhesive systems instead of neat experimental resins, and employed a clinical relevant time for adhesive treatment (ca. 30 s).

Based on previous studies that had reported significant decreases in bond strengths for resin-dentin bonds after short period storage times, [11, 23] we would expect the same in our control groups. However, our results have shown that bonds were stable even after aging, with storage of prepared 0.8 mm² beams [11] and constant changes in water media [52, 55]. The stability of the control group rendered inconclusive the potential benefits of ethanol bonding and 1% chlorhexidine diacetate over the usual procedure. Such stability found in the control group requires, thus, further exploratory analysis. The adhesives in this study were applied using a rubbing action. It has been shown that by agitating adhesive on the surface immediate bond strengths can be increased and stable bonds can be achieved after 12 months of water storage [56]. In this study, the adhesive was applied with rubbing action for all groups, therefore eventual benefits to the stability of the bonds would apply for all groups as well.

It is possible that the stable bonds were a result of an unexpected benefit from the etchant solution used in the present study, (Uni-etch BAC 32%, Bisco Inc., Schaumburg, IL, USA). Recently, Tezvergil-Mutluay e co-authors [57] indicated that benzalkonium chloride (BAC) in concentrations within the range found in the phosphoric acid used in this study binds strongly to demineralized dentin and has the ability to inhibit soluble and matrix bound MMPs. Those authors believe that residues of BAC remain bound to collagen after etching with BAC-containing phosphoric acid, thus providing an inhibitory effect on MMPs [57]. This could explain the stability of the control group over time. For the other groups, the presence of BAC in all groups was an unexpected confounding variable that prevents us from distinguishing the potential benefits of ethanol and chlorhexidine diacetate. Because of this, future studies aiming to evaluate durability of bonds should consider the BAC effect on MMPs when designing the experiment.

The use of AB3 in combination with ethanol (E) or ethanol with 1% chlorhexidine diacetate (E/CHD) solutions resulted in significant reductions in bond strengths after 15 months. This was not observed for Excite. AB3 and Excite have different ethanol content (ca. 20% for

Excite and 49% for AB3). It is possible that when ethanol-containing solutions were used with AB3, an excess residual ethanol may have been present on the surface. This may have had significant effects on the dilution and subsequent polymerization of the monomers [58], thus compromising the stability of the bonds for those groups after 15 months. This warrants further investigation.

The finds of this study confirmed that 1% chlorhexidine diacetate applied for 30 s is also an option as a pretreatment for acid-etched dentin because it does not compromise initial bond strengths and also seems not to compromise long-term stability. A recent study [53] demonstrated that the ethanol wet-bonding technique resulted in preservation of resin-dentin bonds up to 12 months for 5 different resin formulations, graded from the most hydrophobic to the most hydrophilic. In addition, another study challenged the chlorhexidine anti-bond degradation strategy by demonstrating that bonds made to ethanol-saturated dentin with an experimental adhesive were preserved. [41] It seems that yet unknown mechanisms might be involved in the ability of ethanol to preserve bonds, regardless of the presence of chlorhexidine. Perhaps, ethanol wet-bonding technique improves resin infiltration and thus enhances encapsulation of collagen [40], which in turn protect it from the action of MMPs. Additionally, a low residual ethanol content within the adhesive might improve degree of conversion owing to the higher mobility of the reactive components during polymerization, thus resulting in more stable polymer [59]. All this requires further investigation.

Another significant difference found in this study was between two experimental groups for Excite. When dentin was pre-treated with water/1% CHD mix, mean bond strength was significantly lower than when dentin was pre-treated with ethanol/CHD mix. It is risky to credit this difference to either a negative effect of CHD when mixed with water or to a positive effect of ethanol when mixed with CHD because both experimental groups were not different from the control (water) and the ethanol alone group (Table 2). One could argue that chlorhexidine could interfere with the degree of conversion or other property of the adhesive. It has been shown that the addition of 1-5 mass% of chlorhexidine diacetate to experimental resin blends had little effect on the respective degree of conversion, but more significant effects on the reduction of the elastic modulus of some blends were observed [60]. While the negative effect of CHD on the properties of adhesive resins could help explaining some of our results, this should be interpreted with caution because the referred study [60] mixed CHD with the adhesive resin and we used CHD in solution prior to the application of the adhesive. The topic is of interest for further development of combined applications of chlorhexidine and ethanol bonding approaches as a mean to preserve resin-dentin bonds, and warrants further investigation.

Conclusion

The combined use of ethanol bonding with 1% chlorhexidine diacetate did not result in improved stability of resin-dentin bonds after 15-month aging time compared to control groups. Stable bonds were observed with the traditional water wet-bonding and no evident advantage was observed for the use of either ethanol or 1% chlorhexidine diacetate.

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Table 1

Material, composition, manufacturer and lot numbers

Material	Composition	Factory & Bath numbers
Uni-etch 32% BAC	32 % phosphoric acid with benzalkonium chloride (BAC)	Bisco Inc. 0700006350
All Bond 3	PART A: ethanol, MgNTG-GMA (Magnesium Nitro-tri-glycidyl glycidyl methacrylate)	Bisco Inc. 0700005251
	PARTE B: Bis-GMA (Bisphenol A – diglycidyl, ester dimethacrylate); BPDm (bisphenyl, dimethacrylate); HEMA (2-hydroxyethyl methacrylate); photoinitiator, stabilizer	Bisco Inc. 0700005255
Excite	Ethanol, HEMA (2-hydroxyethyl methacrylate); phosphonic acid acrylate, dimethacrylate, silica, fillers, photoinitiator, stabilizer	Ivoclar Vivadent J25791
Acelite All Purpose Body	Bis-GMAE (Bisphenol A ethoxylate – diglycidyl, ester dimethacrylate); TEGDMA: tri-ethyleneglycol, dimethacrylate	Bisco Inc. 0700005779 0700005705 0700005135 0800001576

Table 2

Means of microtensile bond strength values ($\pm$ standard error) in MPa for adhesives All Bond 3 and Excite, considering different surface treatments and storage time. 2-Way ANOVA applied separately for each adhesive.

SURFACE TREATMENT	ALL BOND 3			EXCITE		
	24 hours	6 months	15 months	24 hours	6 months	15 months
WATER	51.07 (3.6) ^{A,a}	57.13 (3.6) ^{A,a}	47.29 (4.4) ^{A,a}	49.51 (5.4) ^{AB,a}	42.10 (5.4) ^{AB,a}	45.51 (6.6) ^{A,a}
CHD/WATER	46.96 (3.6) ^{A,a}	50.69 (3.6) ^{A,a}	46.07 (4.4) ^{A,a}	40.05 (5.4) ^{B,a}	36.78 (5.4) ^{B,a}	40.87 (6.6) ^{A,a}
ETHANOL	59.41 (3.6) ^{A,a}	56.41 (3.6) ^{A,ab}	44.41 (4.4) ^{A,b}	49.67 (5.4) ^{AB,a}	44.56 (5.4) ^{AB,a}	42.48 (5.4) ^{A,a}
CHD/ETHANOL	54.67 (3.6) ^{A,a}	52.17 (3.6) ^{A,ab}	39.58 (4.4) ^{A,b}	53.37 (5.4) ^{A,a}	57.47 (5.4) ^{A,a}	49.55 (6.6) ^{A,a}

Different superscript letters represent values significantly differentes (p<0,05) for All Bond 3. Capitalized letters compare treatments and lower cases compare storage periods.

Different superscript letters represent values significantly differentes (p<0,05) for Excite. Capitalized letters compare treatments and lower cases compare storage periods.

Table 3.1

Distribution of fracture modes (%) of All Bond 3 tested after 24 hours, 6 months and 15 months.

ALL BOND 3												
TREATMENTS	24 HOURS				6 MONTHS				15 MONTHS			
	CD	CC	A	M	CD	CC	A	M	CD	CC	A	M
WATER	32.5	17.5	20	30	42.2	13.3	11.1	33.4	41.7	8.3	29.0	21
ETHANOL	32.4	10.8	10.8	46	23.2	13.9	20.9	41.8	41.6	4.2	37.5	16.6
WATER/CHX	8.8	17.6	50	23.5	25.8	22.5	12.9	38.7	33.3	8.3	33.3	25
ETHANOL/CHX	17.5	7.5	32.5	42.5	32.5	11.6	9.3	46.5	30.7	4.0	42.3	23

Table 3.2

Distribution of fracture modes (%) of Excite tested after 24 hours, 6 months and 15 months.

TREATMENTS	EXCITE											
	24 HOURS			6 MONTHS			15 MONTHS					
	CD	CC	A	M	CD	CC	A	M	CD	CC	A	M
WATER	7.8	13.1	21	57	10.5	7.9	21	60.5	30.7	0	23	46.1
ETHANOL	23.4	20	16.7	40	50	8.8	8.8	32.4	20	3.3	40	36.6
WATER/CHX	13.3	8.9	35.5	42.2	6.5	19.5	26	47.8	16	8	48	28
ETHANOL/CHX	21.6	16.2	5.4	56.7	27.7	25	0	47.2	22.2	18.5	14.8	44.4