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Effect of Oxalate Desensitizer on the Durability of Resin-Bonded Interfaces

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Clinical Relevance

Despite oxalate desensitizer showing a decrease in the rate of resin-dentin bond degradation over time, it compromised the baseline bond strength of etch-and-rinse adhesives to dentin. Further investigation regarding reliability of the combination of oxalate desensitizers and etch-and-rinse adhesives is required prior to implementation of this approach in the clinical routine.

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SUMMARY

Potassium oxalate desensitizers were previously shown to effectively reduce the immediate permeability of resin-bonded dentin. The current study evaluated whether the effect of the combined application of oxalate with etch-and-rinse adhesives interferes with the durability of resin-dentin bonds when using etch-and-rinse adhesives. The bond strength of resin-bonded dentin specimens composed of two-step or three-step etch-and-rinse adhesives (Single Bond, One-Step and Scotchbond Multi-Purpose, respectively)

was tested immediately (24 hours) and after 12 months of water storage. The adhesives were used either according to the manufacturers' instructions (control groups) or after treating acid-etched dentin with a potassium oxalate gel (BisBlock, BISCO, Inc). The treatment of dentin with potassium oxalate was shown to negatively affect the baseline bond strength of resin-bonded dentin specimens, regardless of the adhesive used (p<0.05). After storage, the bond strength of the resin-bonded interfaces was significantly reduced for all the tested groups (p<0.001). Nevertheless, the rate of decreasing bond strength was significantly lower for oxalate-treated specimens than for the controls (p<0.05).

INTRODUCTION

It is generally accepted that the efficiency and quality of adhesive bonding to dentin depends on a homogeneous and complete hybridization between the exposed collagen fibrils and resin polymers. One of the current approaches for achieving this goal is to keep the demineralized dentin saturated in water, so that the nanospaces between the collagen fibrils can be expanded, theoretically making them more prone to the uptake of resin.[1] Conversely, when using the water-wet bonding technique, the water content on the acid-etched dentin surface makes the hybridization of dentin with hydrophobic resins impractical, forcing the manufacturers to include hydrophilic monomers in dental adhesive formulations.[2]

Among many critical issues, excess residual water within demineralized dentin has been shown to interfere with the conversion of resin monomers into polymers[3-4] and cause phase separation between the hydrophobic and hydrophilic components of dental adhesives,[5-6] which results in the formation of porous hybrid layers.[7-8] Inopportunely, during and after adhesive polymerization, an outward fluid flow of dentinal tubules can be consistently observed.[8-9] This chain of events (the formation of a porous hybrid layer and hybrid layer/adhesive layer permeability) compromises the perfect sealing of acid-etched dentin,[10-11] thus increasing the prevalence of microleakage and/or post-operative dentin sensitivity.[12]

The application of acidic solutions of potassium oxalate has been used in clinical dentistry to desensitize dentin. Potassium oxalate desensitizers react with ionized calcium in dentin to form insoluble calcium oxalate crystals, which can occlude the dentinal tubules.[13-15] When applied on acid-etched dentin, calcium oxalate crystals tend to form only in the tubules, leaving the dentin surface unobstructed and available for bonding with dental adhesives.[16] Thus, the use of oxalate during bonding procedures could enhance solvent evaporation by decreasing the amount of water entrapped within these adhesives, allowing for better control of the adequate moisture and facilitating the penetration of adhesives into wet demineralized dentin.[17-18]

Although recent studies have shown that oxalate desensitizers seem not to compromise early bond strength (over short periods, [24 hours]) of relatively neutral adhesives to dentin, none of these studies tested the effect of this combination over time.[16-17] The current study evaluated whether the effect of the combined application of oxalate with etch-and-rinse adhesives interferes with the durability of resin-dentin bonds when using two- and three-step etch-and-rinse adhesives. The null hypothesis tested was that potassium oxalate does not alter the bond strength of etch-and-rinse adhesives to dentin, either when tested immediately after the restorative procedures or after one year of aging in water.

METHODS AND MATERIALS

Tooth Selection and Preparation

Thirty extracted, unerupted human third molars stored at 4°C in saline containing 1% thymol, were selected for the current study. The teeth were used within six months of extraction.

This study protocol was approved by the Human Assurance Committee of the Piracicaba School of Dentistry, University of Campinas, São Paulo, Brazil.

Crown segments were prepared by removing the occlusal enamel and roots of selected teeth, using a slow-speed diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) under water-cooling. The pulpal tissue was carefully removed with a pair of small forceps to avoid crushing the predentin. The dentin surface was further abraded with 600-grit silicon carbide paper until a remaining dentin thickness of 1.5 ± 0.2 mm was achieved from the ground surface to the highest pulp horn. The resulting crown segments were glued to Plexiglass slabs (1.8 x 1.8 x 0.7 cm) using viscous cyanoacrylate (Zapit, Dental Ventures of American, Corona, CA, USA), which also covered the entire peripheral cementum. Each Plexiglass slab was penetrated by a short length of 18-gauge stainless steel tubing, which ended flush with the top of the slab. This tube permitted the pulp chamber to be filled with a neutral pH fluid consisting of phosphate buffered saline supplemented with 0.02% sodium azide in order to inhibit bacterial growth during the storage period.

Specimen Preparation and Storage for Microtensile Bond Testing

Three etch-and-rinse adhesives were used in the current study: two two-step systems–Adper Single Bond 2 (SB, 3M ESPE, St Paul, MN, USA) and One-Step (OS, BISCO, Inc, Schaumburg, IL, USA) and one three-step system–Adper Scotchbond Multi-Purpose (MP, 3M ESPE). These materials were used either according to the manufacturers' instructions (control groups) or after treatment of the acid-etched dentin with potassium oxalate gel (BisBlock, BISCO, Inc) (Table 1). Briefly, the potassium oxalate gel was applied on the acid-etched dentin for 30 seconds and abundantly rinsed off with water (60 seconds). The enamel margins were re-etched for 15 seconds and rinsed thoroughly. After the potassium oxalate application, the adhesives were applied according to the manufacturers' instructions. The surfaces were checked to ensure complete coverage with the adhesives before light-activation (Degulux Soft-Start, Degussa Dental, Hanau, Germany), which was performed under a power density of 500 mW/cm^2. The specimens were covered with four layers (± 1.0-mm thick) of a resin composite (Z-250, 3M ESPE) to build up an approximately 4 mm high "crown." Each resin increment was light-cured for 40 seconds (Degulux Soft-Start).

After storage in distilled water at 37°C for 24 hours, each tooth was longitudinally sectioned into two halves: one-half was prepared for immediate testing in tension, while the other half had the exposed dentin surface protected with a varnish, and only then was it stored in phosphate buffered saline supplemented with 0.02% sodium azide at 37°C for one year. The exposed dentin was covered with nail varnish to avoid direct contact with water, as this can accelerate the decrease in bond strength for microtensile specimens subsequently obtained from that area.[19] Just before the microtensile testing (immediately or after one year's water storage), each hemi-tooth was sectioned across the bonded interface in both the x and y directions, using a diamond impregnated disk (Labcut 1010, Extec, Corp, Enfield, CT, USA) to obtain at least eight resin-bonded beams with a cross-sectional area of approximately 0.8 mm^2. These specimens were individually fixed to a custom-made testing jig with cyanoacrylate glue and were subjected to microtensile testing at crosshead speed of 0.5 mm/minute until failure (Model 4411, Instron Corporation, Canton, MA, USA). The fractured specimens were sputter-coated with gold/palladium and examined with a scanning electron microscope (JEOL-5600 LV, Tokyo, Japan) at 15 Kv. The failure modes were classified as cohesive failures in bonding resin and/or in resin composite (CR), in dentin (CD), in the hybrid layer (CHL) or as mixed failures (M).

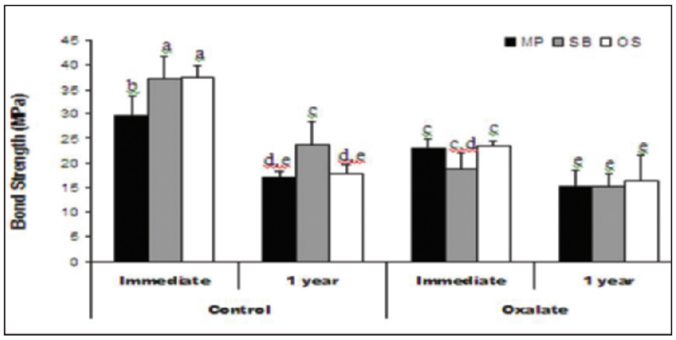

Figure 1. *The mean bond strength and standard deviations for all tested groups, number of specimens (5 hemi-teeth)/experimental condition. MP = Adper Scotchbond Multi-Purpose system; SB = Adper Single Bond 2 system and OS = One-step system. The height of the bars is the bond strength mean; half-brackets indicate plus one standard deviation. Groups identified wtih the same case letters did not differ statistically (*p*>0.05).*

Table 1: *Composition and Application Mode of Materials Employed in This Study*

Material (Manufacturer)		Composition (Batch #)	Application
Gel Potassium Oxalate Desensitizer	**BisBlock** (BISCO, Inc, Schaumburg, IL, USA)	Oxalic acid, potassium salt and water (0500009787)	Apply for at least 30 seconds on the acid etched dentin, rinse and leave moist for bonding. If enamel is present, re-etch the enamel, then rinse and leave moist for bonding.
Adhesives	**Adper Scotchbond Multi-Purpose** (3M ESPE, St Paul, MN, USA)	Primer: aqueous solution of HEMA and a polyalkenoic acid copolymer. Adhesive: Bis-GMA, HEMA, initiators (5PH)	Apply primer and dry gently for five seconds. Apply adhesive and light cure for 10 seconds.
	Adper Single Bond (3M ESPE, St Paul, MN, USA)	Bis-GMA, HEMA, dimethacrylates, polyalkenoic acid copolymer, initiators, water and ethanol (4BC)	Apply two consecutives coats, dry gently for two to five seconds and light cure for 10 seconds.
	One Step (BISCO, Inc, Schaumburg, IL, USA)	HEMA, BPDM, initiator and acetone (0600001383)	Apply two coats of adhesive and dry for 10 seconds. If the surface is not glossy, apply additional coats and dry.
Resin Composite	**Filtek Z-250** (3M ESPE, St Paul, MN, USA)	Bis-GMA, UDMA, Bis-EMA, initiator and zircon/silica filler	Insert four 1.0-mm layers and light cure them, individually, for 40 seconds.

Abbreviations: BIS-GMA: Bisphenol A diglycidyl ether dimethacrylate; Bis-EMA: Bisphenol A polyethylene glycol diether dimethacrylate; HEMA 2-Hydroxyethyl methacrylate, BPDM: Biphenyl dimethacrylate; UDMA: urethane dimethacrylate.

Statistical Analysis

The experimental unit in the current study was the hemi-tooth, as half of the specimens were tested immediately and the remaining half was tested after one year. A three-way ANOVA and post hoc Bonferroni test were used to analyze the effects of "adhesives" (MP, SB and OS), "dentin treatment" (control and with oxalate) and "storage time" (immediate vs one year) on bond strength to dentin. The Student's *t*-tests were used to compare the effect of water storage on the reduction of bond strength in control versus oxalate-treated specimens within each used adhesive. In this analysis, the adhesives were not compared. The statistical significance was preset at α=0.05.

RESULTS

The mean bond strength and standard deviations for all the tested groups are shown in Figure 1. During an immediate analysis, the treatment of dentin with potassium oxalate was shown to negatively affect the resin-dentin bond strength of dentin specimens, regardless of the adhesive employed ($p<0.05$). After 12 months of water storage, the bond strength of resin-bonded interfaces was significantly reduced for all the tested groups ($p<0.001$). The decrease in bond strength values was significantly higher for the control groups than for the experimental groups ($p<0.05$); that is, for those where the dentin was treated with potassium oxalate. The percentage of bond strength reduction for the oxalate-treated groups varied between 18%-30%, while for the controls, it ranged between 36%-52% (Table 2).

When tested immediately, the most prevalent fracture pattern observed for the control specimens was mixed failure (M); while for the oxalate-treated specimens, the failure modes were nearly equally distributed among mixed (M), cohesive in bonding resin (CR) and within the hybrid layer (CHL) (Table 3). After water storage, the most frequent mode of failure observed was CR, regardless of the dentin treatment (Table 3).

Table 2: *Reduction (%) of the Bond Strength (as a function of the baseline results) for Control Versus Oxalate-treated Specimens After One-year Storage in Water*

Adhesives	Control Groups	Oxalate-treated Groups
MP	41% A	31% B
SB	36% a	18% b
OS	52% [a]	30% [b]

*Analysis in row: Different case letters indicate statistically significant differences between the Control- and Oxalate-treated groups within the factor "adhesive" (p<0.05; Student's t-test). Comparisons between adhesives were not performed. **MP**= Adper Scothbond Multi-Purpose system; **SB**= Adper Single Bond 2 system and **OS**= One-step system.*

DISCUSSION

The application of potassium oxalate to acid-etched dentin significantly affected the initial performance of the tested adhesives. In addition, although the decrease in bond strength has been observed for most of the specimens submitted to prolonged storage in water, the rate of bond strength reduction for the experimental groups, which were treated with potassium oxalate, was significantly lower compared with that of the controls (Table 2). Therefore, these results do not support the full acceptance of the null hypothesis that potassium oxalate does not affect the bond strength of etch-and-rinse adhesives to dentin either when tested immediately or after one year of aging in water.

According to previous studies, free fluoride ions released from fluoride-containing adhesives may potentially interact with calcium to form spherical loosely bound calcium fluoride crystals.[20-21] These spherical calcium fluoride crystals are probably derived from dissolution of the calcium oxalate crystals that are also formed by the application of potassium oxalate to acid-etched dentin. Calcium oxalate crystals are located deeper inside the dentinal tubules and their solubility is sensitive to pH changes. That is why adhesives with a relatively low pH are thought to create conditions that favor the formation of calcium fluoride crystals. The point is that the calcium fluoride crystals may be located more superficially in the dentinal tubules, and they could somehow interfere with the adhesives' infiltration/polymerization,[20-21] compromising formation of the hybrid layer.

The adhesives used in the current study were intentionally chosen because they exhibit low fluoride con-

Table 3: *Percentage Distribution of Cohesive Failure Mode*

	Control								Oxalate							
	Immediate				One Year				Immediate				One Year			
	CR	CD	CHL	M	CR	CD	CHL	M	CR	CD	CHL	M	CR	CD	CHL	M
MP	18	0	30	52	54	0	46	0	24	0	38	38	26	0	32	42
SB	7	0	35	57	0	0	77	23	38	0	29	32	59	0	14	18
OS	38	3	0	59	64	0	4	32	32	4	46	18	50	0	27	23

***CR**: cohesive in bonding resin and/or resin composite, **CD**: cohesive in dentin, **CHL**: within the hybrid layer and **M**: mixed. **MP**= Adper Scothbond Multi-Purpose system; **SB**= Adper Single Bond 2 system and **OS**= One-Step system.*

tent,[20-21] a relatively high pH (3.3 for MP, 3.6 for SB and 4.5 for OS) (3M ESPE/BISCO, Technical Product profile) and, mainly, because they have been reported not to affect the immediate bond strength to dentin when used along with oxalates.[16-17,21] Two of the adhesives tested in the current study (SB and OS) were exactly those that, in the previously-mentioned studies, demonstrated not having their bond strength altered by use of an oxalate desensitizer.[20-21] The lack of consistency between the previous and current results encourages the authors of this study to ponder the combined application of oxalate desensitizer and etch-and-rinse adhesives to acid-etched dentin as not being safely reproducible.

In a parallel study, the authors of the current study observed that, when applied to acid-etched dentin under similar conditions to that of this study, all of the tested adhesives (OS, SB and MP) had their microhardness significantly compromised by the previous application of BisBlock (Silva & others, unpublished data). Thus, although the oxalate solution had been thoroughly rinsed before the adhesives' application, residual oxalic acid may have remained and reacted with calcium, causing crystal precipitation on the dentin surface, which, in turn, could disturb polymerization of the adhesives and, ultimately, compromise their bonding performance.

Another concern relates to the pH of the tested oxalate desensitizer. BisBlock (monopotassium monohydrogen oxalate) has a pH ranging from 1.5-1.8.[23] Since this acidic oxalate was applied after the dentin had been acid-etched with 35% phosphoric acid, it might be possible that, despite producing calcium crystals that helped to obliterate the dentinal tubules, this desensitizer agent had also caused additional etching of the dentin, thus increasing its extent of demineralization. Studies indicated that bond strength to over-etched dentin can be markedly reduced[19,24-25] and this is likely related to the increased discrepancy between the extent of demineralization and the depth of penetration of the adhesive monomers.[25]

Nonetheless, despite BisBlock having affected the initial bond strength to dentin, it seemed somehow to slow down the rate of bond strength reduction over time (Figure 1 and Table 2). The decrease in bond strength to oxalate-treated specimens was significantly lower when compared to that exhibited for the controls, regardless of the tested adhesive. Significant reductions in bond strength to dentin have been observed after middle- to long-term water storage.[26-28] This bond strength reduction has been attributed to water sorption effects, which swell the resin network and reduce the frictional forces between polymer chains, causing a decrease in their mechanical properties.[29] Hydrophilic resins, such as those used in the current study, are highly prone to absorbing water,[30-32] having their intrinsic strength damaged immediately by plasticization,[29,33] and eventually by hydrolytic degradation. The presence of calcium oxalate crystals partially blocking the fluid transudation across the dentinal tubules might have prevented the adhesives from prematurely absorbing water, thus decelerating mechanical disruption by the plasticizing effects of water.

In a previous study, Vachiramon and others[34] reported the detrimental effect of oxalate desensitizer on the bond strength to dentin that was bonded with Single Bond and stored for three months under simulated pulpal pressure. TEM micrographs showed that the hybrid layers formed on oxalate-treated dentin were heavily impregnated with silver deposits. Unfortunately, in that study,[34] the baseline (immediate) bond strength values, both to the controls or the oxalate-treated specimens, were not determined. The authors of the current study assume that the baseline bond strength values were not reported in that study, because the specimens' preparation to perform the initial microtensile test would not allow the authors to check the middle-term effects of pulpal pressure on the durability of resin-bonded dentin. Nevertheless, it is possible that, if the authors had the chance to prepare additional specimens to determine the initial bond strength of oxalate-treated specimens, they could have determined whether the bonding performance of Single Bond was immediately affected by the application of BisBlock and if the initial bond strength of oxalate-treated specimens differed significantly from that of the corresponding specimens stored for three months under simulated pulpal pressure.

In theory, hybrid layers created with three-step etch-and-rinse adhesives that indicate the separate application of a resin that is relatively more hydrophobic than acid-etched primed-dentin, may be less susceptible to absorbing water than that formed when using simplified two-step etch-and-rinse adhesives. However, the results of the current study showed that the decrease in long-term bond strength for specimens bonded with a three-step etch-and-rinse adhesive (MP) was not significantly lower than the bond strength reduction exhibited for specimens bonded with simplified two-step etch-and-rinse adhesives (SB and OS) (Figure 1). Adhesives that indicate a separate application of hydrophobic comonomers over primed dentin may be more resistant to the diffusion of water that comes from external sources than to the diffusion of water that is present in the dentinal tubules and/or in intertubular dentin. In a similar fashion to that of two-step etch-and-rinse adhesives, the hydrophilic components of the primer solutions of three-step etch-and-rinse adhesives[35] respond to formation of the hybrid layer in acid-etched dentin that is intentionally saturated with water. Thus, the resin layer, which is in direct contact with the dentin and dentinal tubules, using the current adhesives, is essentially a hydrophilic structure prone to permeation by intratubular dentinal fluid. Fluid movement across resin-bonded dentin presumably travels from fluid-filled dentinal tubules around the interface between resin tags surrounding the dentin matrix, then through fluid-filled porosities in the overlying adhesive or across the interface between the overlying adhesive and the top of the hybrid layer.[11] If fluid passes through the adhesive, it may accumulate on top of the hybrid layer and interfere with coupling to resin composites.[8-9]

Despite the current protocol being performed with extracted teeth, the bonding procedures were done while the pulp chamber was filled with an aqueous solution that was not removed during water storage. This guaranteed that the dentin was kept hydrated. This occurrence, in concert with the fact that the use of three-step etch-and-rinse adhesives did not prevent bond strength reduction over time, leads the authors of the current study to speculate that the residual water present in the dentinal tubule can be more critical to the long-term degradation/stability of the hybrid layers than water derived from sources external to the tooth. Thus, for HEMA-rich hydrophilic adhesives, such as those tested in the current study, water absorption from dentin is expected to occur basically through an osmotic mechanism.[35] Moreover, considering that the oxalate-treated specimens exhibited a lower rate of bond strength reduction over time, which is likely due to dentinal tubules obliteration, it seems to be imperative that, to perfectly seal dentin, the dental adhesives need to be greatly resistant to the effects of dentinal water, which has not been demonstrated by using the current systems.

Water in acid-etched dentin has been shown to be largely replaced by ethanol. This allows the use of more hydrophobic resin blends for dentin bonding.[1] The authors of the current study have recently found that the permeability of resin-dentin bonds can be significantly reduced if the acid-etched dentin is saturated with ethanol and subsequently bonded with hydrophobic adhesives.[11,36] The ultimate goal of the "ethanol-wet bonding"[1] is to infiltrate the interfibrillar spaces and dentinal tubules with hydrophobic dimethacrylate resins that do not absorb much water. In addition, the infiltration of relatively hydrophobic monomers in ethanol-filled interfibrillar spaces has been shown to produce similar or higher initial bond strengths to dentin when compared to those achieved by the infiltration of hydrophilic monomers in water-saturated acid-etched dentin.[1,37-38] These results were obtained when dentin specimens were bonded in the absence of fluid contamination from dental pulp, as the ethanol wet-bonding protocol was found to be very technique-sensitive in the presence of water.[38]

Recently, a study showed that it is possible to achieve high bond strengths to dentin when experimental hydrophobic adhesives are used with the adjunctive use of potassium tetroxalate prior to the dehydration of dentin by ethanol saturation.[39] It was suggested that, as the dentinal tubules were patently blocked with oxalate crystals, the fluid contamination of the dentin surface could have been prevented during the application of hydrophobic adhesives,[39] thereby forming better quality resistant hybrid layers. Future studies should be conducted to check whether the application of oxalates to acid-etched dentin saturated with ethanol and bonded with hydrophobic adhesives can form resin-dentin bonds that are more stable and durable.

CONCLUSIONS

Within the limits of the current study, it may be concluded that, despite oxalate desensitizer being shown to contribute to the reduction of resin-dentin bond degradation over time, it compromised the baseline bond strength of etch-and-rinse hydrophilic adhesives to dentin. For this reason, the combined application of oxalate desensitizers and etch-and-rinse hydrophilic adhesives to acid-etched dentin should be further investigated before it can be fully recommended for clinical use.

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