

Permeability of Modified Dentinal Surfaces

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ABSTRACT

Objectives: The aim of this study was to investigate the sealing ability of three adhesives bonded to modified dentinal surfaces after short and long storage periods.

Methods: Three adhesives were used in this study; two-step etch-and-rinse “Adper Single Bond 2”, two-step self-etch “AdheSE” and one-step self-etch “G-Bond” adhesives. Modifications of the dentinal surface was performed by application of adhesives after oxalate application, application of adhesive after oxalate application to sodium hypochlorite (NaOCl)-deproteinized dentinal surface, compared to control groups (application of adhesive without any surface pretreatment). Dentinal sealing was investigated by measuring dentin permeability, using a fluid transport apparatus, after two storage periods; 24 hours and 2 months.

Results: After 24 hours, the control group bonded with “Adper Single Bond 2” without any surface treatment had the highest permeability (25.3%) followed by “G-Bond” (16.2%) and finally “AdheSE” (11%). Significant reductions in permeability values were observed on application of oxalate in conjunction with both “Adper Single Bond 2” and “AdheSE” (11.7% and 2.6% respectively). Further significant reductions in permeability values of those two adhesives were noted when the combined NaOCl and oxalate pretreatments were used (5.7% and 0.8% respectively). Permeability means for all groups increased after storage for 2 months.

Conclusions: Dentin surface deproteinization plus oxalate application produced the best dentin sealing for two step adhesives.

Keywords: Etch-and-rinse adhesives, self-etching adhesives, oxalate-desensitizing agent, NaOCl-deproteinizing agent, dentin permeability.

Clinical Significance: Although newly developed adhesive resins have attempted to improve dentin sealing, biodegradation of resin-dentin bonds over time jeopardize the durability of resin composite restorations with subsequent recurrent caries, discoloration and hypersensitivity.

INTRODUCTION

Adhesive dentistry should effectively restore the peripheral seal of dentin after enamel and/or cementum removal. Many resin composite restorations have been associated with postoperative sensitivity, impaired bond durability and

occasional pulpal inflammation. This mainly occurs due to improper dentinal sealing, with subsequent movement of dentinal fluid in the dentinal tubules and bacterial products permeation through or around adhesive materials. Dentin adhesives should adhere firmly to the dentin and seal

dentinal tubules as well as does enamel or cementum^[1-5].

The assumption that adhesive-sealed dentin is impermeable to water movement has resulted in using adhesives to reduce cervical hypersensitivity via resin-occlusion of exposed dentinal tubules. Similarly, sealing of tooth preparations for indirect restorations with dentin adhesives has been advocated in fixed prosthodontics that involves the preparation of vital teeth. Of particular concern is whether these adhesives can be effectively employed for eliminating dentinal fluid movement in freshly exposed, vital deep dentin that is close to pulp horns, where the contribution of a positive pulpal pressure is expected to be high^[6].

Dentin bonding has been evolving rapidly to include the use of hydrophilic adhesive monomers to ensure that contemporary dentin adhesives are compatible with the intrinsic wetness of acid-etched dentin. Thus, adhesive monomers have been modified to contain both hydrophilic and hydrophobic moieties. By virtue of these modifications, adhesives have an increased potential for water sorption especially, contemporary highly hydrophilic adhesives^[6-9]. Water sorption by hydrophilic resin monomers within both the hybrid and adhesive layers has been considered to contribute to the degradation of resin-dentin interfaces over time^[4, 7, 10, 11].

The use of oxalate desensitizers has been suggested in an attempt to reduce the hydraulic conductance through the dentinal tubules. However, this effect may be temporary as surface crystals of calcium oxalate are solubilized by oral fluids and may be removed by daily brushing^[12-18]. Adhesive resin comonomers have also been advocated for occluding exposed dentinal tubules. However, they usually form a weak thin layer on top of the dentinal surface which can be also lost by daily brushing. The loss of the surface layer of resin during oral hygiene activities should not permit a return to dentin sensitivity if residual resin

tags remain occluding the tubules. Unfortunately, defective hybridization as well as polymerization shrinkage of resin produce leaky resin tags^[19]. Furthermore, resin tags may hydrolytically degrade over time, causing the development of a gap between the tag and the surrounding tubule, allowing sufficient fluid shifts to cause dentin sensitivity^[15, 20, 21]. Thus, in an attempt to improve bond durability and reduce dentin sensitivity, oxalate occlusion of dentinal tubules was combined with resin occlusion. This combination was more effective than either treatment alone^[15, 22].

Due to the hydrophilic nature of collagen, the hybrid layer has a hydrogel-like behavior, absorbing and releasing water^[8]. This may be responsible for the degradation of resin-dentin interfaces^[9, 23-27, 28]. Thus, application of sodium hypochlorite to acid-etched dentin was suggested to remove collagen fibrils and their bound water from the bonded interface.

The aim of the current study was to investigate methods of reduction of water flux through dentinal tubules, modification of the hydrophilic content of the dentin substrate and the use of a more hydrophobic adhesive in an attempt to improve its sealing capacity. This may have an impact on improved bond durability with subsequent reduction in hypersensitivity and increase in the survival rate of resin composite restorations. The null hypothesis tested was that the different dentin surface treatments, used in this study, do not affect the sealing ability of the three tested adhesives.

MATERIALS AND METHODS

Permeability Test

A total of ninety freshly extracted, sound human unerupted third molars were selected and used in this study. All soft tissues were removed and the teeth were stored in distilled water at 4°C for no longer than one month prior to specimen preparation.

Ninety coronal deep dentin discs were prepared using a diamond disc (K6974

Komet, Germany) mounted on a conventional speed straight handpiece (W & H, Austria) under continuous water irrigation. Deep dentin was selected as it is considered the most permeable dentin due to its dense large diameter tubules^[29]. Crowns of the selected teeth were separated from their root segments. The crown segments were ground gradually from the pulpal side, perpendicular to the long axis of the tooth until the pulp horns completely disappeared (to avoid variation in sample morphology). Then, grinding was performed on the occlusal side until the thickness of the discs was reduced to 1 mm (+ 0.1mm).

Both surfaces (pulpal and occlusal) of each dentin disc were abraded with 600 grit silicon carbide paper under water

irrigation for 30 seconds, to create a standardized smear layer. The prepared discs were stored in distilled water until the bonding procedure was performed within 3 days.

Permeability apparatus:

A fluid transport apparatus was used to test the permeability of the dentin discs (Fig. 1a,b). The apparatus was constructed according to the device designed by Pashley and Galloway^[30], with slight modifications. The apparatus was connected to an electric pump with a rubber tube to provide controlled pulpal pressure (15 cm H₂O),^[2, 3, 29] which was regularly checked using a sphygmomanometer.

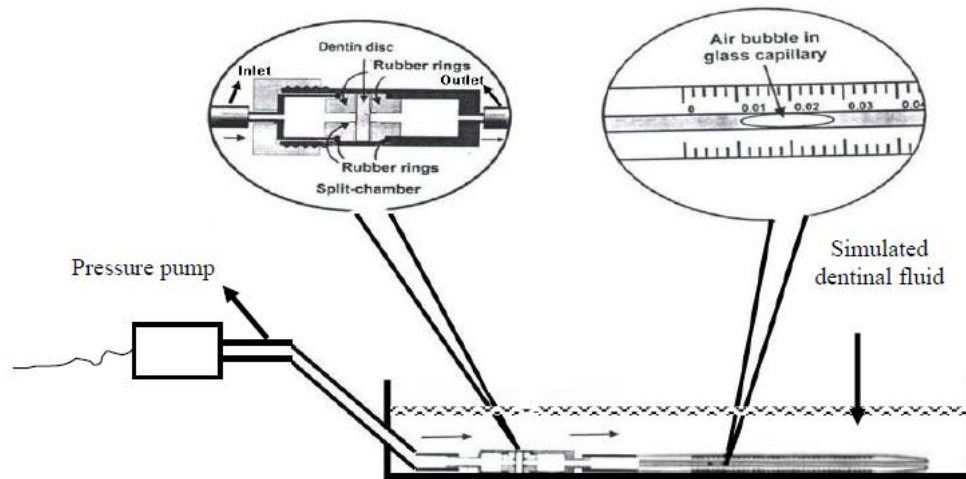


Figure 1(a): Diagram of fluid transport apparatus assembly

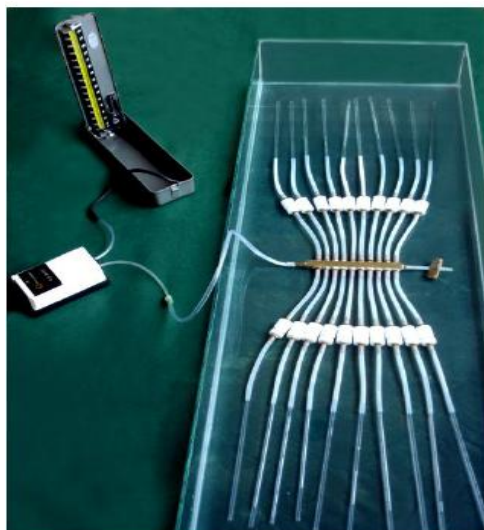


Figure 1(b): Fluid transport apparatus assembly

The fluid transport apparatus included ten pairs of split-Teflon chamber devices, each consisting of two parts, female and male parts which were screwed together (Fig. 1a). A pair of identical rubber "O" rings, with a central hole (0.5 cm in diameter), were used to limit the tested surface area of each dentin disc. The apparatus was immersed in a simulated dentinal fluid (Dr. Peter Shellis' artificial saliva, Table 1). Care was taken to fill all the inner parts of the assembly with the simulated dentinal fluid to displace all air bubbles.

Mechanism of measuring the dentin permeability:

Dentin permeability was measured by tracing the displacement of an air bubble within the glass capillaries, inserted into the outflow of the split-chamber devices (Fig. 1 a), using a millimeter scale [3, 13, 15, 30, 31].

A- Measurement of Pre-treatment Fluid Filtration (baseline value):

All discs were etched on both sides with 35% phosphoric acid gel for 15 seconds and then rinsed with water. After all the discs were mounted into their split chambers, the air bubble location was determined for every specimen and recorded.

Pressure was applied for one hour, and the movement of the air bubble was redetermined and recorded. Then, the linear displacement of the air bubble in the horizontally positioned glass capillary tube was calculated. Dentin permeability varies considerably among different teeth, therefore, discs with extremely high or low permeability were excluded.

Baseline fluid flow (Q) was derived from the linear displacement of the air bubble, depending on the volume of the pipette, using the following equation:-

$Q = \text{displacement} \times \text{cross sectional area of the pipette.}$

Permeability was expressed in terms of fluid filtration (Jv) [22, 32, 33] where:

$$J_v = Q/At$$

Jv = fluid filtration rate in $\mu\text{l cm}^{-2} \text{ min}^{-1}$

Q = fluid flow in μl .

A = dentinal (or O ring central hole) surface area in cm^2

t = time in minutes.

Baseline fluid filtration represents the maximum fluid flow of each specimen and was assigned a value of 100% permeability.

B- Surface treatment of the specimens:

After recording the baseline fluid filtration, the discs were removed from the split chambers. The occlusal side of each disc was polished using 600 grit silicon

carbide paper for 30 seconds, under water irrigation to recreate a uniform smear layer. All samples were randomly divided into three groups (30 each) according to the adhesive system used; 1) Etch-and-rinse adhesive (Adper Single Bond 2); 2) Two-step, self-etch primer/adhesive (AdheSE); 3) One-step, self-etch adhesive (G-Bond). Each group was randomly subdivided into three subgroups (10 specimens each) according to the surface pretreatment performed: 1) Adhesive alone (Control- no surface pretreatment); 2) Oxalate pretreatment; 3) NaOCl and oxalate pretreatment. The compositions of the materials used in this study are presented in table 1.

For the control specimens, each adhesive system was applied according to its manufacturer's instructions without any surface pretreatment (Table 1).

For the specimens in the oxalate pretreatment subgroups, 3% monopotassium monohydrogen oxalate desensitizing agent (Bisblock, Bisco Inc, USA) was applied on the occlusal dentinal surface for 30 seconds as follows; after acid-etching in the Adper Single Bond 2 group or after self-etch priming in the AdheSE group or applied on smear layer-covered dentin before application of G-Bond adhesive. Then, the treated dentin surfaces were rinsed with water and were blot-dried, except for the specimens of the AdheSE group, they were air-dried only. Subsequent adhesive application was performed according to manufacturers' instructions.

For the specimens receiving sequential NaOCl and oxalate treatment, 5.25% NaOCl dentin deproteinizer was applied on the occlusal dentinal surface for 2 minutes, after acid-etching in the Adper Single Bond 2 group and on smear layer-covered dentin in the AdheSE and G-Bond groups. The dentin surfaces of all specimens were rinsed thoroughly for 30 seconds then were blot-dried. This was followed by oxalate application and finally the adhesive application in the Adper Single Bond 2 group. In the AdheSE group, the self-etch

primer was applied to the deproteinized dentin surface followed by oxalate application and finally the adhesive was applied. In the G-Bond group, the oxalate was applied on the deproteinized smear layer-covered dentin then the adhesive was finally applied. All specimens were then stored in simulated dentinal fluid at 37°C for 24 hours and for 2 months.

C- Measurement of Post-treatment Fluid Filtration:

Each treated disc was remounted in its specific chamber and the fluid filtration of bonded dentin, after each storage period, was measured exactly as the baseline measurement was performed. The dentin permeability of each resin-bonded specimen of all groups after each storage period was obtained using the following equation [31, 32]:

$$\% P = \frac{\text{Fluid filtration rate of resin-bonded dentin}}{\text{Baseline fluid filtration rate of etched dentin}} \times 100$$

This represents the permeability exhibited by the resin-bonded dentin relative to its maximum acid etched value, with each specimen serving as its own control.

Statistical Analysis:

Data management and analysis were performed using Statistical Package for Social Sciences (SPSS) vs. 17. Data were summarized using means and standard deviations. Comparisons between groups were done using three-way analysis of variance (ANOVA) with repeated measures as one factor, surface treatment as the second factor and adhesives as the third factor. All p-values are two-sided. P-values ≤ 0.05 were considered significant.

RESULTS

Permeability measurements:

There were three different adhesives tested: 1) The etch-and-rinse primer/adhesive blend, Adper Single Bond 2; 2) the two-step, self-etching primer + neat adhesive, AdheSE; 3) the single-step, single bottle, self-etching adhesive, G-Bond (Table 1). There were three different surface treatments with each adhesive group. The pretreatment variables were: 1) control- no pretreatment, just adhesive application; 2) pretreatment with 3% potassium oxalate for 30 sec, followed by adhesive application; 3) pretreatment with 5.25% NaOCl for 2 min, rinsed, followed by oxalate treatment, followed by bonding.

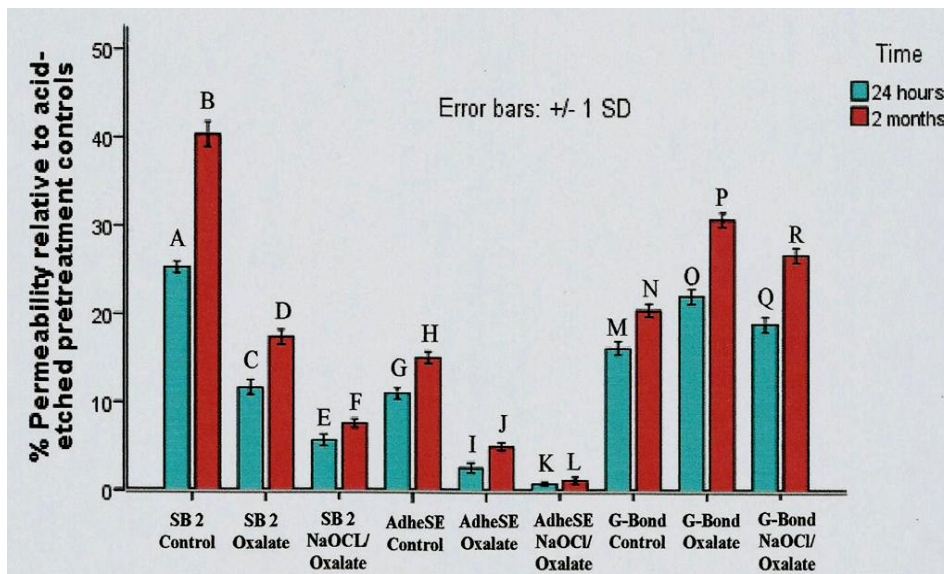


Figure 2: Post-treatment permeability of dentin specimens treated with Single Bond 2, AdheSE, or G-Bond, with no treatment (controls), oxalate, or NaOCl/oxalate pretreatments, measured after 24 hours and 2 months. Bars identified by different pairs of letters are significantly different at p < 0.05. Only AdheSE pretreated with NaOCl and oxalate showed minimal increase in permeability over 2 months. The post-treatment permeability in that group was almost zero. Values are means ± SD, n = 10.

In the control groups for Single Bond 2, where no pretreatment of the dentin surface was made except for acid-etching dentin with 37% phosphoric acid for 15 sec, followed by rinsing with water for 10 sec and applying two layers of Adper Single Bond 2, the initial 24 h permeability was relatively high (Fig. 2, Table 2). That is, the bonded dentin permitted $25.3 \pm 0.7\%$ as much fluid flow as acid-etched, uncovered controls had permitted. Within 2 months of storage in a buffer, the permeability of Single Bond 2 bonded specimens increased significantly ($p < 0.05$) to $40.4 \pm 1.4\%$ of control values.

When acid-etched dentin was treated with 3% potassium oxalate for 30 sec and then rinsed with water, briefly dried and then moist bonded with Single Bond 2, the 24 h permeability of the bonded specimens was only $11.7 \pm 0.8\%$ as much fluid flow as the acid-etched, uncovered controls had been. Two months later, the permeability value had increased ($p < 0.05$) to $17.4 \pm 0.8\%$.

When acid-etched dentin surfaces were treated with 5.25% NaOCl for 2 min, rinsed for 30 sec, then treated with 3% potassium oxalate for 30 sec, rinsed and then bonded with Single Bond 2, the 24 h permeability values were only $5.7 \pm 0.6\%$ as much as was the acid-etched control value (100%). The 2 month permeability values were not much changed from the 24 h values (Fig. 2, Table 2).

When AdheSE primer was used on smear layer-covered dentin, followed by application of the neat adhesive, the 24 h permeability values $11.0 \pm 0.6\%$ was only half that of the Adper Single Bond 2 control values (Fig. 2, Table 2). After storage for 2 months, the AdheSE values increased slightly to $15.1 \pm 0.7\%$.

When the AdheSE primer-covered dentin was treated with 3% potassium oxalate for 30 sec, before application of the neat adhesive, the 24 h permeability values were only $2.60 \pm 0.59\%$ of acid-etched controls. After 2 months, that value increased to $5.00 \pm 0.42\%$.

However, when smear layer-covered dentin was treated with 5.25% NaOCl (2 min), followed by AdheSE primer, then 3% potassium oxalate, followed by the neat adhesive, the 24 h permeability value was only $0.80 \pm 0.24\%$, the lowest permeability value measured in the experiment, indicating near-perfect sealing. Two months later, the permeability value only increased to $1.20 \pm 0.37\%$, the lowest 2 month permeability value in the study.

When G-Bond adhesive was bonded to untreated control smear layer-covered dentin, the 24 h permeability values were $16.2 \pm 0.8\%$. After storage for 2 months, the G-Bond values increased to $20.5 \pm 0.7\%$.

However, when the smear layer-covered dentin was treated with 3% potassium oxalate and then bonded with G-Bond, the post-treatment permeability value, $22.1 \pm 0.9\%$, was higher than in the no-treatment control condition, $16.2 \pm 0.8\%$ (Fig. 2 and Table 2), suggesting that oxalate treatment decreased the self-etching adhesive's ability to seal dentin. Furthermore, two months later, the permeability value had increased ($p < 0.05$) from $22.1 \pm 0.9\%$ to $30.8 \pm 0.8\%$.

Similarly, when the smear layer-covered dentin surface was treated sequentially with NaOCl and potassium oxalate, prior to bonding, the G-Bond 24 h permeability values were again, higher than the untreated control group (compare 18.9 ± 0.9 to $16.2 \pm 0.8\%$ - Fig. 2, Table 2) with further increase after 2 months to $26.8 \pm 0.9\%$, indicating that G-Bond did not seal dentin very well.

DISCUSSION

The permeability results of this study indicated that when no surface pretreatment was applied, the two-step etch-and-rinse Single Bond 2 adhesive exhibited the greatest post-treated residual dentin permeability compared with the two tested self-etch adhesives. These results were in agreement with other reports [5, 32-34]. The high permeability of Adper Single Bond 2 adhesive may be attributed to smear layer

removal prior to bonding which opens up the dentinal tubules and exposes the collagen matrix after rinsing off the etchant, increasing dentinal fluid flow.

Furthermore, the high permeability of Single Bond 2 bonds may also be attributed to incomplete resin penetration into the deeply etched dentin due to the use of a separate aggressive phosphoric acid etch causing over-etching [35]. Moreover, as the two-step etch-and-rinse Single Bond adhesive utilized a rinsing step and was

applied according to manufacturer's instructions, using the wet bonding technique, incomplete water removal from the deeply etched dentin might have resulted in the "overwetting phenomenon" [36]. After etching with phosphoric acid and rinsing with water, dentin has a water content of 70 vol. % [9]. The single bottle system includes a mixture of hydrophilic and hydrophobic groups as well as solvents that allow more water seepage than neat, solvent free adhesives like AdheSE adhesive (Table 1).

Table 1: Materials used, their composition, application procedure and manufacturer.

Material	Composition	Application Procedure	Manufacturer
Adper Single Bond 2 <i>(Two-Step Etch-and-Rinse)</i>	Etchant: 37% phosphoric acid gel. Adhesive : BIS-GMA*, HEMA*, dimethacrylate, water, ethanol, copolymer of polyalkenoic acid and polyitaconic acid, colloidal silica nanofiller, camphorquinone.	Etchant: Apply etchant for 15 seconds, rinse for 10 seconds and blot dry excess water leaving a glistening surface. Adhesive: Apply 2 consecutive coats of adhesive with gentle agitation for 15 seconds, air thin gently for 5 seconds and light cure* for 10 seconds.	3M ESPE, Dental Products, St. Paul, MN, U.S.A.
AdheSE <i>(Two-Step Self-Etch)</i>	Self-etching primer: Phosphonic acid acrylate, dimethacrylate, initiators, stabilizers, water. Adhesive: HEMA, dimethacrylate, highly dispersed silicon dioxide, initiators and stabilizers.	Self-etching primer: Apply self-etching primer for 30 seconds with brushing action, air thin strongly for 5 seconds. pH = 1.4 Adhesive: Apply adhesive, air thin gently and light cure* for 10 seconds.	Ivoclar-Vivadent AG, FL-9494 Schaan / Liechtenstein
G-Bond <i>(One-Step Self-Etch)</i>	4-MET [□] , phosphoric ester monomer, UDMA [⊙] , acetone, water, silica nano-filler, camphorquinone.	Apply one coat of adhesive on dentin surface. Leave undisturbed for 10 seconds, strong air drying for 5 seconds. pH = 2. Light cure* for 10 seconds.	GC Corp. Tokyo, Japan
Bisblock	3% monopotassium monohydrogen oxalate desensitizing solution, pH = 2.5.	Apply on dentinal surface for 30 sec.	Bisco Inc. Schaumburg IL, U.S.A.
Dr. Peter Shellis ⁷ artificial saliva	CaCl ₂ . 2H ₂ O 0.103 g/L MgCl ₂ . 6H ₂ O 0.109 g/L KH ₂ PO ₄ 0.544 g/L Sodium azide 2.23 g/L HEPES [▲] buffer (acid) 2.24 g/L		Arcos Organics New Jersey, U.S.A. Mallinckrodt Baker, Inc. Phillipsburg, NJ, U.S.A.

*BIS-GMA : Bisphenol A glycidyl dimethacrylate.

◦ HEMA : hydroxyethyl methacrylate.

□ 4-MET : 4 methacryloxyethyl trimellitic acid..

⊙ UDMA : urethane dimethacrylate monomer.

▲ HEPES : N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid.

* Mini LED, 1250 mW/cm², Satelec, Acteon.

Since water is the main causative factor in degradation of resin-dentin interfaces, complete or partial blockage of the dentinal fluid flow from the underlying dentin may improve dentinal sealing. This was evident by the significant decrease in permeability results obtained using Single Bond 2 adhesive, in conjunction with oxalate pretreatment, in agreement with Yiu et al. [22]. The oxalate desensitizer was used after acid etching which might have resulted in subsurface precipitation of calcium oxalate crystals occluding the dentinal tubules. At the same time subsurface

precipitation of calcium oxalate crystals did not interfere with resin penetration into the dentinal surface. Furthermore, the resin tags may seal the calcium oxalate crystals in place, avoiding the solubilizing effect of oral fluids. Such combined treatment was advised in several research reports [14, 16-18, 20, 30, 36].

The permeability results showed further significant decreases in dentin permeability when Single Bond 2 adhesive was used in conjunction with oxalate, after deproteinization with NaOCl, indicating that this combination produced better dentin

sealing. This may be due to elimination of most of the exposed collagen within the dentin surface. As collagen has a low surface energy and a high water content, its removal would improve the wettability of the dentin surface. The collagen fibrils also contain the endogenous proteases of dentin that are responsible for the slow hydrolysis of hybrid layers^[9,23]. The use of NaOCl destroyed and solubilized those matrix metalloproteinases (MMPs). Consequently, the possibility of incomplete resin penetration into etched dentin with subsequent defective hybridization, responsible for deterioration of dentin bonding, may be eliminated or reduced^[38-42]. These results require rejection of the test null hypothesis that different surface treatments do not affect the sealing properties of adhesive systems.

Deproteinization of etched-dentin might have resulted in an increase in the number and diameter of opened dentinal tubules leading to an increase in the number and diameter of resin tags which may improve the bonding efficiency^[39, 41, 43, 44]. **Prati et al.** called this resin-rich, collagen-poor resin-dentin interface a "reverse hybrid layer"^[45].

The cumulative effect of phosphoric acid and NaOCl treatment on dentin resulted in larger dentinal tubules diameters which might have made it difficult for oxalate crystals to completely occlude the dentinal tubules, making the interface more permeable to fluid flow. It is desirable to have less porous dentin to provide better possibility for dentinal sealing. However, using Single Bond 2 after etching, NaOCl and oxalate treatment might have resulted in resin penetration and integration between the calcium oxalate crystals, reducing permeability of Single Bond 2 bonded dentin surfaces to their lowest values (Table 2).

The introduction of the self-etch technique eliminated many technique-sensitive factors related to the etch-and-rinse technique^[35]. This involves elimination of the separate etch and rinse

steps and lowers the water content of self-etched dentin compared to etch-and-rinse adhesives. The weak acidity of self-etch adhesives only modified the smear layer and plugs rather than completely removing them, leaving dentin permeability very low and providing better sealing^[30, 46-50]. Furthermore, self-etch adhesives superficially demineralize the underlying dentin, providing a continuous hybrid layer with less unpolymerized hydrophilic monomers. Several previously published studies support those results^[37, 48, 50, 51]. Incomplete resin penetration is less likely due to simultaneous etching and priming of dentin to the same depth via the same acidic monomer which eliminates the need to re-wet dentin or re-expand shrunken collagen^[42, 49, 52].

However, the permeability results of the current study showed that when no surface pretreatment was applied, self-etch adhesives created resin-dentin bonds that were still permeable to fluid flow. This may be attributed to the fact that these adhesives contain increased concentrations of hydrophilic resin monomers to facilitate their penetration into the hydrophilic dentin surface. This would, in turn, increase their capacity for water sorption. Also, AdheSE and G-Bond contain 25- 35% water to reduce viscosity and to ionize the acidic monomer to be able to demineralize the underlying dentin^[53-55]. Residual water retained within the bonded interface contributes to increased permeability of adhesives to fluid flow. This was in agreement with several studies that have reported that self-etch adhesives behave as semi-permeable membranes after polymerization^[5, 21, 35, 49, 56].

The permeability of self-etch adhesives may be also attributed to the presence of the calcium salts of the acidic monomers and calcium phosphates, derived from demineralization of dentin (as no rinsing was performed), as well as uncured hydrophilic resin monomers. All these factors decrease the local water concentration, creating an osmotic pressure

gradient that initiates water movement from the underlying dentin through the cured self-etch adhesives [10, 56, 57].

It is worth noticing that the permeability of the two-step self-etch AdheSE adhesive was significantly lower than the other two tested adhesives. The improved sealing capacity of the neat AdheSE adhesive may be attributed to the presence of a separate layer of solvent-free, relatively hydrophobic, less permeable

bonding resin placed over primed dentin. This layer might have limited the diffusion of water from the underlying dentin through the hybrid layer, decreasing water sorption and providing better dentinal sealing [5, 33, 34, 56]. Furthermore, it was reported that the application of a separate neat adhesive as a separate step may provide sufficient time for proper water/solvent evaporation and proper resin penetration [58].

Table 2: Permeability means and standard deviations (SD) exhibited by each adhesive system after different surface treatments at the two testing periods.

Adhesive System	Surface Treatment	24 hours		2 months	
		Mean	SD	Mean	SD
dper Single Bond 2	No pretreatment	25.3 ^f	0.7	40.4 ^f	1.4
	Oxalate + Adhesive	11.7 ^E	0.8	17.4 ^e	0.8
	NaOCl/oxalate/ adhesive	5.7 ^C	0.6	7.6 ^c	0.5
AdheSE	No pretreatment	11.0 ^D	0.6	15.1 ^d	0.7
	Oxalate + Adhesive	2.60 ^B	0.59	5.00 ^b	0.42
	NaOCl/oxalate/ adhesive	0.80 ^A	0.24	1.20 ^a	0.37
G-Bond	No pretreatment	16.2 ^F	0.8	20.5 ^f	0.7
	Oxalate + Adhesive	22.1 ^H	0.9	30.8 ^h	0.8
	NaOCl/oxalate/ adhesive	18.9 ^G	0.9	26.8 ^g	0.9

Values represent the post-treatment values of dentin permeability expressed as a percent of untreated acid-etched controls. For instance, a value of 25.3 ± 0.7% means that bonding with Single Bond reduced dentin permeability 100-25.3= 74.7 percent, leaving the bonded dentin 25.3% as permeable as the untreated control value for that disc. Groups identified by different letters are significantly different at $p < 0.05$. Values are mean ± SD (%), n = 10.

The permeability test results (Table 2) showed that when oxalate was applied following the self-etching AdheSE primer application, significantly lower permeability with better dentin sealing was obtained. This may be attributed to the potential interaction that might have occurred between the oxalate solution and the dissolved calcium ions present within the resin (since they are not washed away) producing insoluble oxalate crystals. These crystals might have occupied the microscopic spaces within the hybrid layer and penetrated into the tubules to participate in resin tag formation providing better integration and sealing. Furthermore, these crystals were precipitated within the resin layer so they were more protected from the dentinal fluid than those precipitated within the tubules that were in direct contact with the fluid.

The lowest dentin permeability was obtained when the smear layer-covered dentin was treated with NaOCl, followed by self-etching AdheSE primer, then oxalate

application was performed. Smear layer compositions are similar to the originating tissue (50% vol. minerals and 30% vol. collagen) [40, 42]. The application of NaOCl to the smear layer would eliminate its collagen phase which would facilitate the penetration of the self-etch primer through water-filled channels between particles of smear layer enlarging them to reach the underlying mineralized dentin more easily [42].

Part of the demineralized dentin collagen may be in a denatured unstable state which is highly sensitive to hydrolysis [43, 40]. Based on this assumption, NaOCl might have removed the organic content of smear layer as well as some organic content of the underlying dentin, thus decreasing the hydrophilic collagen within the dentinal surface with subsequent decrease in water sorption. The effect of NaOCl on smear layer-covered dentin was supported by other studies [42, 59].

Single-bottle self-etch adhesives are characterized by their increased

hydrophilicity and decreased viscosity as they contain high concentration of HEMA and water/solvent combinations. The presence of the highly hydrophilic HEMA was advisable to improve wetting and spreading of adhesives on dentin as well as for maintaining dimethacrylates in solution and preventing phase separation [31, 49, 60- 62].

On the other hand, HEMA and other comonomers have been recently recognized to lower the vapor pressure of water and so prevent its complete removal from the adhesive during bonding and promote residual water in unstable soft hydrogels within both hybrid and adhesive layers [49, 60, 61]. Furthermore, such highly hydrophilic polymers are capable of imbibing large amount of water from the underlying dentin. Thus, absorbed water remains entrapped within the resin-dentin interface, that gradually increases over time. Such water plasticizes the polymers and degrades their mechanical properties. This may facilitate degradation of hydrophilic polymers (collagen and resin) that lowers the durability of resin-dentin interface [31, 49, 60, 63].

In an attempt to overcome problems created by HEMA, recent HEMA-free one-step self-etch adhesives, containing less hydrophilic comonomers, have been introduced. The omission of HEMA from the adhesive blends has been considered advantageous in preventing water sorption [31, 49, 60, 61]. However, the absence of HEMA necessitates higher water/solvent content to maintain components in solution. The high acetone content (40%) of the G-Bond may lower the viscosity of the solution, enhancing the penetration of the bonding agent into the demineralized collagen-rich dentin matrix, and may lower the surface tension of water due to its "water chasing" effect [49, 61].

However, rapid acetone evaporation, allows increases in water concentration, causing phase changes in the comonomer blend. The recommended application time may not be enough for allowing water to diffuse upwards and be evaporated from the

partially polymerized adhesive layer. Rapid acetone evaporation may lower the temperature of the comonomers, decreasing monomer diffusion rates [61].

Due to the partial solubility of some resinous compounds (like UDMA), hydrophobic and hydrophilic-rich phases form. Phase separation within the HEMA-free adhesive may result in the separation of free water, not completely evaporated, within dimethacrylate-rich adhesive resin resulting in a porous bonded interface. Thus, vigorous air-drying of adhesive, prior to polymerization, according to the manufacturer's instructions is thought to be very important for removal of these water blisters [31, 48, 59, 60, 61].

On the other hand, the permeability results of the current study showed that G-Bond, when applied without any surface pretreatment, provided a permeable bonded interface that was even more permeable than that provided by the two-step HEMA-containing self-etch adhesive AdheSE, although it was applied using vigorous drying [31,64]. The permeability of G-Bond adhesive may be attributed to the fact that vigorous air drying may induce evaporative water flux from dentin, allowing dentin water movement into the adhesive layer bonded to dentin. Water movement through the polymerized adhesive matrix may further generate additional pathways for water penetration, increasing water uptake.

In an attempt to improve dentinal sealing, acidic potassium oxalate was applied to smear layer-covered dentin, based on the assumption that G-Bond adhesive could produce chemical bonding with inorganic-rich substrate due to the presence of 4-methacryloxyethyl trimellitate (4-MET) in its composition. Oxalate was not preceded by an etching step as this would increase permeability due to all problems associated with the etch-and-rinse technique, mainly smear layer removal and over-etching.

Unfortunately, oxalate pretreatment resulted in a significant increase in permeability when compared to dentin discs

treated with G-Bond adhesive without any surface pretreatment. This may be attributed to the fact that application of the slightly acidic (pH = 2.5) oxalate solution on smear layer, transforms the dentin surface from an acid-labile to an acid-resistant structure, probably due to the removal of the original smear layer by etching and its replacement by an acid-resistant layer of calcium oxalate crystals and calcium phosphate crystals^[30]. Calcium ions needed to form calcium oxalate crystals are obtained from calcium in the mineral phase of the smear layer. Although, these insoluble crystals probably reduce dentin permeability, they are highly resistant to demineralization which created an obstacle for adhesive penetration into the dentinal surface especially when using the weakly acid G-Bond adhesive.

The self-etching conditioning effect of G-Bond adhesive might have resulted in loose oxalate crystals within the bonded interface. These loose crystals might have decreased the wettability as well as the bonded surface area. Consequently, the bonding efficiency of G-Bond may have been adversely affected. This was in agreement with the results of *Yiu, et al.*^[14], who reported that loose crystals within the bonded interface may also act as stress-raisers which would, in turn, create debonding at lower stresses.

However, in the current study when NaOCl treatment preceded oxalate application in the G-Bond group, the dentin permeability decreased significantly when compared to oxalate pretreatment alone. This may be attributed to the production of a relatively porous smear layer due to removal of its organic content. Such effect might have, somehow, facilitated the resin penetration into dentin after precipitation of the calcium oxalate crystals with slightly improved micro-mechanical retention. Thus, it may be suggested that the interaction of G-Bond with dentin depends mainly on micromechanical interlocking rather than chemical bonding. Furthermore, reducing the collagen content might have

reduced the water content of the bonded interface.

Effect of aging on bond durability

Degradation of resin-dentin bonds by aging may be attributed to continuous fluid transudation from the underlying dentin into the bonded interface, under positive pulpal pressure. Consequently, fluid may continue to pass into the bonded interface via leaky resin tags, defective hybridization and porosities within the adhesive. On the other hand, another time-dependent effect is the slow hydrolytic action of the endogenous matrix metalloproteinases such as MMP-2,-8,-9 and-20. These endogenous enzymes have collagenolytic and telopeptidase activity which may further contribute to the degradation of the hybrid layer^[23, 65-71]. What used to be regarded as hybrid layer degradation due to elution of unpolymerized resins may have been due to slow degradation of collagen by endogenous matrix proteases^[72]. Thus, storage for two months produced time-dependent water sorption and subsequent hydrolytic degradation that was reflected by significant increases in permeability results of all subgroups of this study (Table 2).

Degradation of both collagen and hydrophilic resin might have occurred, but to a lesser extent, when calcium oxalate crystals were formed within the bonded interface. This may be attributed to less fluid transudation from the occluded dentinal tubules and/or sealed hybrid layers (supported by permeability results, Table 2).

Collagen removal by NaOCl pretreatment in this study resulted in a more durable resin-dentin interfaces, which were reflected by significant decreases in permeability results when comparing 2-month oxalate alone pretreatment results with 2-month NaOCl and oxalate pretreatment results. Thus, resin retention based on resin infiltration of collagen fibrils has been shown to be unstable over time^[41, 42, 44, 65-71, 73].

CONCLUSIONS

Based on the results of this study, it can be concluded that:

1. None of the tested adhesives could provide an impervious seal of deep dentin surfaces after 24-hour storage period (except for AdheSE using NaOCl + oxalate pretreatment).
2. Storage for two months increased the permeability of the bonded interfaces of all subgroups with varying degrees reaching its minimum with NaOCl + oxalate pretreatment of AdheSE group.
3. Bonding technique plays a critical role in the sealing capacity of different adhesives.
4. The two-step self-etch adhesive (AdheSE), used in this study, was the best sealing adhesive material.
5. The HEMA-free adhesive (G-Bond), used in this study, did not provide better sealing of dentin surfaces.
6. Oxalate application was able to improve the sealing of the dentin surfaces when used in conjunction with the two-step etch-and-rinse and the two-step self-etch adhesives, but impaired dentinal sealing when used with the one-step self-etch G-Bond adhesive.
7. Dentin deproteinization may improve resin-dentin adhesion and reduce bonded-interface permeability. This emphasizes the negative role of the collagen fibril matrix in bond degradation and reduced durability of resin-dentin bonds.

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