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RESEARCH ARTICLE

IN VITRO COMPARISON BETWEEN THE EFFECT OF GLUTARALDEHYDE-CONTAINING DESENSITIZERS AND ONE-STEP SELF-ETCHING ADHESIVES ON DENTIN PERMEABILITY IN TEETH PREPARED FOR FULL-COVERAGE RESTORATIONS

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ABSTRACT

Objective: The aim of this study was to evaluate and compare in vitro the effectiveness of one-step self-etching adhesive and two glutaraldehyde-containing desensitizers in reducing dentin permeability in extracted human premolar teeth prepared for full-coverage restorations using a dye penetration test.

Materials and Methods: Thirty -six intact human premolars were prepared in a standardized manner for full coverage restorations. The exposed coronal dentin was completely covered by nail varnish except for a finish line and a ring area of 2mm coronal dentin. Afterward the specimens were randomized into 4 groups(n=9) according to dentin treatment: group 1, non-treatment samples; group 2, dentin treated with Gluma®; group 3, treated with Systemp®; Group 4, treated with Tetric® N-Bond Self-Etch. After treatment, they were placed in freshly prepared 2% aqueous methylene blue dye for 12 hours and then sectioned in the buccolingual and mesiodistal direction to assess the dye penetration under a stereomicroscope. Dye penetration was recorded according to the ratio of the maximum linear dye penetration length in millimeters to the total dentin thickness. The results were statistically analyzed using one way ANOVA. The Tukey's post hoc test was used to compute multiple pairwise comparisons that identified differences among groups at $p < 0.05$.

Results: All dentin treatment agents significantly reduced dentinal permeability versus control. There were significant differences in permeability among dentin treatment agents ($p < 0.001$). The self-etching adhesive group showed the lowest permeability—this was statistically different from both desensitizers. There were no significant differences in permeability among desensitizers ($p = 0.98$).

Conclusions: Within the limitations of this study, the dentin treatments revealed different sealing abilities. The one step self-etch adhesive Tetric®N-Bond Self-Etch exhibited the most favorable dentin sealing properties against dye penetration versus both glutaraldehyde-containing desensitizers Gluma® and Systemp®.

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INTRODUCTION

Dentin is a permeable substance that allows exchange between dentin and pulp through dentinal tubules (Mjör, 2009; Mjör *et al.*, 2001). This is essential to support the vitality of dentin and its capability to react with physiologic and pathologic stimuli (Roberson *et al.*, 2006). However, when dentin is exposed in oral cavity by disease or operative procedures, the same path that allows passage of nutrients and impulses will allow passage of irritants (exogenous substances such as bacteria and toxins) from the oral cavity to pulp. This jeopardizes the biological health of the dentin-pulp complex. Moreover, any stimulus on the exposed dentinal surface such as

cutting, drying, pressure changes, osmotic shifts, or changes in temperature will create fluid movements in open dentinal tubules that stimulates mechanoreceptor nerve endings present inside tubules near the pulp evoking pain and patient discomfort according to hydrodynamic theory—the most commonly accepted theory to explain dentin hypersensitivity (Brännström *et al.*, 1986; Borges *et al.*, 2012).

Preparing the tooth to receive full coverage indirect restoration will expose a large area of vital deep dentin that contains large numbers of wide dentinal tubules open directly to the pulp. Moreover, the prepared tooth is then covered temporarily by poor sealing provisional restoration until the definitive restoration is fabricated in the dental laboratory. This is due to the poor sealing ability of temporary cements (Farah and Alzaky, 2015). These factors making this prepared tooth more

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prone to penetration of exogenous substances and bacteria from the oral cavity (Whitworth *et al.*, 2002). There are over several hundred bacterial species in the mouth (Berkiten *et al.*, 2000). This infection may move toward the pulp and evoke intense inflammatory changes in the pulp-dentin complex that leads to pulpal pathology with subsequent pulpal death (Bergenholtz *et al.*, 1981). In addition, excessive fluid movement inside dentinal tubules under hydrostatic and osmotic pressure can cause stimulation of nerve endings and lead to dentin hypersensitivity and patient discomfort and pain.

The use of dentin desensitizing agents for reducing dentin permeability and hypersensitivity after full-coverage preparation is an effective protective treatment for dentin. These agents occlude or reduce the diameter of the patent dentinal tubules by plugging dentinal tubules either by protein or mineral salt precipitates (Bartold, 2006). Other dentin treatments have been also used successfully to seal superficial dentinal tubules either by dentin adhesive (dentine bonding agents). These impregnate the acid-etched or conditioned dentin surface by resin to form after polymerization an impermeable hybrid layer (Maghrabi *et al.*, 2011; Hu *et al.*, 2010). Or by lasers which can also be used to alter the dentin surface morphology by melting and solidification/recrystallization of the dentin surface and dentinal smear layer to make the dentin surface impermeable (Liu *et al.*, 1997).

Glutaraldehyde-containing desensitizers are an example of protein precipitating agents (Ishihata *et al.*, 2012). Glutaraldehyde reacts with serum albumin in the dentinal fluid to precipitate serum albumin and septum formation. This blocks or reduces the diameter of the dentinal tubules (Schüpbach *et al.*, 1997; Arrais *et al.*, 2004; Qin *et al.*, 2006). The efficacy of glutaraldehyde-containing desensitizers in reducing dentin permeability and hypersensitivity has been shown in many reports (Olusile *et al.*, 2008; Patil *et al.*, 2015; Stewardson *et al.*, 2004). Unfortunately, glutaraldehyde and its vapors are known to be irritants, and cytotoxic (Eyüboğlu *et al.*, 2015; Stein *et al.*, 2001). Glutaraldehyde can damage the gingiva after long-term exposure. Moreover, it may cause a vascular pain (with pulse) in teeth (Jalalian *et al.*, 2009).

Dentine bonding agents has been used to seal dentinal tubules since the early 90s and have been proven by many reports to reduce dentin permeability (Pashley *et al.*, 1992; Sahin *et al.*, 2012). These total-etch systems are considered a gold standard in dentin adhesives. Unfortunately, application of Total-Etch Adhesives is technique sensitive and over-wetting and over-drying of the acid-etched dentin interfere with optimal hybrid layer formation (hybridization) (Frankenberger *et al.*, 2000). Misuse of commonly used phosphoric acid may have a necrotizing effect on the periodontal soft tissues as described in few clinical reports (Akman *et al.*, 2005). This is especially common during full coverage indirect restorations or procedures near the gingival tissue. This increases the probability of contact between acid with the gingiva. Moreover, the use of strong phosphoric acid in these systems—especially in vital deep dentin—may cause postoperative sensitivity. This is because the strong acid denatures the collagen and irritates the pulp. Second, strong acid can completely remove the smear layer and leave open dentinal tubules with continues transudate

of dentinal fluid due to positive pulpal pressure. This will cause water-filled blisters to form along the adhesive interface. Third, strong acid can demineralize a thicker layer of dentine and the resins cannot reach the same depth leaving voids in the hybrid layer (Hashimoto *et al.*, 2000; Bouillaguet *et al.*, 2001; Pashley, Michelich and Kehl, 2003).

Recently, one-step self-etch adhesives have been developed to eliminate the separate etching and rinsing steps and to combine all components (etching agent, primer and bond) in a single bottle. This makes application less technique-sensitive and time-consuming. Moreover most of these systems contain a weak acid to etch the dentine surface (Tay *et al.*, 2001). This barely damages the pulp and prevents sensitivity because the weak acid has a limited demineralization and can only modify the smear layer leaving smear plugs which account for 86% of the total resistance to fluid movement in deep dentine (Pashley *et al.*, 1978). Moreover the monomer penetrates the same depth of the acid so that there is little hiatus between the sound dentin and the polymerized resin (Delannée *et al.*, 2013). Therefore, it is a good option to reduce dentin permeability and hypersensitivity following full-coverage tooth preparation.

Therefore, the purpose of this in-vitro study was to evaluate and compare in vitro the effectiveness of one-step self-etching adhesive and two glutaraldehyde-containing desensitizers in reducing dentin permeability using a dye penetration test. The tested null hypothesis is that there is no significant difference between the above-mentioned dentin treatments in terms of dentin permeability reduction.

MATERIALS AND METHODS

I. Specimen preparation

Thirty six freshly extracted intact human maxillary premolar teeth were collected and kept in distilled water at room temperature until use. The root of each tooth was embedded to facilitate manipulation in cold-cure acrylic resin 1 mm apical to cemento-enamel junction utilizing a ring mold obtained from 19 mm-diameter cylindrical polyethylene pipe. The teeth were then prepared for full coverage restoration in a standardized manner using a rotary diamond-cutting instrument in a high-speed hand-piece with water spray coolant. The preparations were performed with a flat occlusal reduction, a 1 mm radial shoulder finish line and 1.5 axial reductions.

Each tooth surface was coated with an acid-resistant protective nail varnish (-) except for the finish line and a 2-mm ring area from the axial wall dentin occlusal to the finish line. This were done by placing 2 mm wide strips of paper around the circumference of the prepared tooth finish line and axial wall and securing it with adhesive tape before nail varnish painting. After the varnish dried completely, the adhesive tape was removed with fine tweezers to leave the finish line and a 2mm ring of prepared axial wall dentin uncovered by the varnish.

II. Dentin treatment procedures

One-step self-etch adhesive and two glutaraldehyde-containing desensitizers were used in the current study. The

self-etching adhesive was Tetric N-Bond Self-Etch. The glutaraldehyde-containing desensitizers were Gluma and Systemp (Table 1).

millimeters to the total dentin length. The value of the dye penetration assigned to each specimen was the average of the scores of dye penetration recorded from the four measurement points.

Table 1. Description of dentin treatment agents used in this study

Brand	Type	Main composition*	Manufacturer	Lot No.
Gluma	Desensitizer	Glutaraldehyde, 2-Hydroxyethyl methacrylate HEMA	Heraeus Kulzer GmbH, Hanau (Germany)	010208
Systemp	Desensitizer	Glutaraldehyde, Maleic Acid, PolyethyleneGlycol Dimethacrylate	Ivoclar/ Vivadent AG, Schaan, Liechtenstein	S54589
Tetric N-Bond Self-Etch	Dentin Bonding Agent	Bis-acrylamide derivative, bismethacrylamidedihydrogenphosphate, amino acid acrylamide, hydroxyalkylmethacrylamide, water, nano sillicafillers, initiators and stabilizers	Ivoclar/ Vivadent AG, Schaan, Liechtenstein	R71125

*According to the information provided by the manufacturer.

The specimens were randomly divided into four groups of 9 specimens, and each group received a different dentin treatment as follows:

Group (1): (control group) had no treatment;
 Group (2):the dentin surface was treated with Gluma;
 Group (3):the dentin surface was treated with Systemp.
 Group (4): the dentin surface was treated with one Tetric N-Bond Self-Etch.

The dentin treatment agents were strictly applied according to the manufacturer's instruction. For Gluma and Systemp, the prepared tooth was rinsed with water, gently air-dried, and desensitizer was applied once to the dentin surface. Once the surfaces were completely coated, they were left undisturbed for 60 seconds, and carefully air-dried until the fluid film disappeared and the surface was no longer shiny. They were then thoroughly rinsed with water for 20 s. For Tetric N-Bond Self-Etch, the adhesive was directly applied with adequate amount using the Viva Pen after the prepared tooth was rinsed with water and gently air-dried. Once the surfaces were completely coated, we continued to brush the product into the entire surface For 30 s. This was then air-dried with a strong stream of air until there is no longer any movement of the material and light polymerized with a light-emitting diode (LED) visible light-polymerizing unit (Smart Lite ® Max LED Curing Light Dentsply/ Caulk, Milford, Del, USA) for 10 s and then thoroughly rinsed with water again for 20 s.

Next, the specimens were kept in freshly prepared 2% aqueous methylene blue solution at $23 \pm 1^\circ\text{C}$ for 12 hours and then removed and gently washed with tap water for 10 minutes. The nail polish was scratch removed, and the specimens were then blotted dry.

III. Dye penetration Assessment

Each tooth was sectioned longitudinally in the buccolingual and mesiodistal directions with a diamond disc (Super Diamond; NTI-Kahla GmbH, Germany) under dry conditions. This sectioning provided four separate measurement points per specimen. These were examined under a stereomicroscope (Hamilton, Hamilton international s.r.l. Lazio, Italy) at an original magnification of $20\times$. The extent of the dye penetration was scored by a single operator using the ratio of the maximum linear methylene blue-dye penetration length in

IV. Statistical Analysis

After verifying the normality of the results with the Shapiro-Wilkes test, data was analyzed with a one-way analysis of variance (ANOVA). Tukey's post hoc test was used for pairwise comparisons between the means when the ANOVA tests were significant. The level of significance was $p < 0.05$. The statistical analysis was performed with statistical SPSS 20.0 software (Statistical Package for Scientific Studies Inc., Chicago, IL, USA).

RESULTS

The means and standard deviations of dye penetration score of each treatment group are shown in Figure 1. Dye penetration scores were normally distributed for all groups of dentin treatments as assessed by Shapiro-Wilk's test ($p > 0.05$). And there was homogeneity of variances, as assessed by Levene's test of homogeneity of variances ($p = .592$). Statistical analysis (1-way ANOVA) revealed the significant effects of the dentin treatments ($(F(3,32) = 2809.302, p < 0.001)$) on the amount of dye penetration. Most control specimens showed dye leakage almost reaching the pulp chamber (Figure 2), and the specimens treated with desensitizers (Fig.3 and 4) and adhesive system (Figure 5) demonstrated dye penetration to a lesser extent. A Tukey post-hoc test for all pairs of dentin treatments revealed that the dye penetration score was statistically significantly lower in specimens treated with an adhesive system ($0.1056 \pm 0.1067, p < 0.001$) as well as in both desensitizers Gluma ($0.8633 \pm 0.235, p = 0.039$) and Systemp ($0.8611 \pm 0.0267, p = 0.024$) versus the control group (0.8933 ± 0.02236). There were no statistically significant differences between the Gluma and Systemp groups ($p = 0.997$), however the adhesive system group was significantly different from both desensitizers groups ($p < 0.001$).

DISCUSSION

In the current study effectiveness of one step self-etch adhesive and two Glutaraldehyde-containing desensitizers in reducing dentin permeability in teeth prepared for full-coverage restoration were investigated using dye penetration test which has been one of the earliest acceptable method for dentin permeability assessment (Bitter, 1990; Al-Turki & Akpata, 2002; Mjör, 2009).

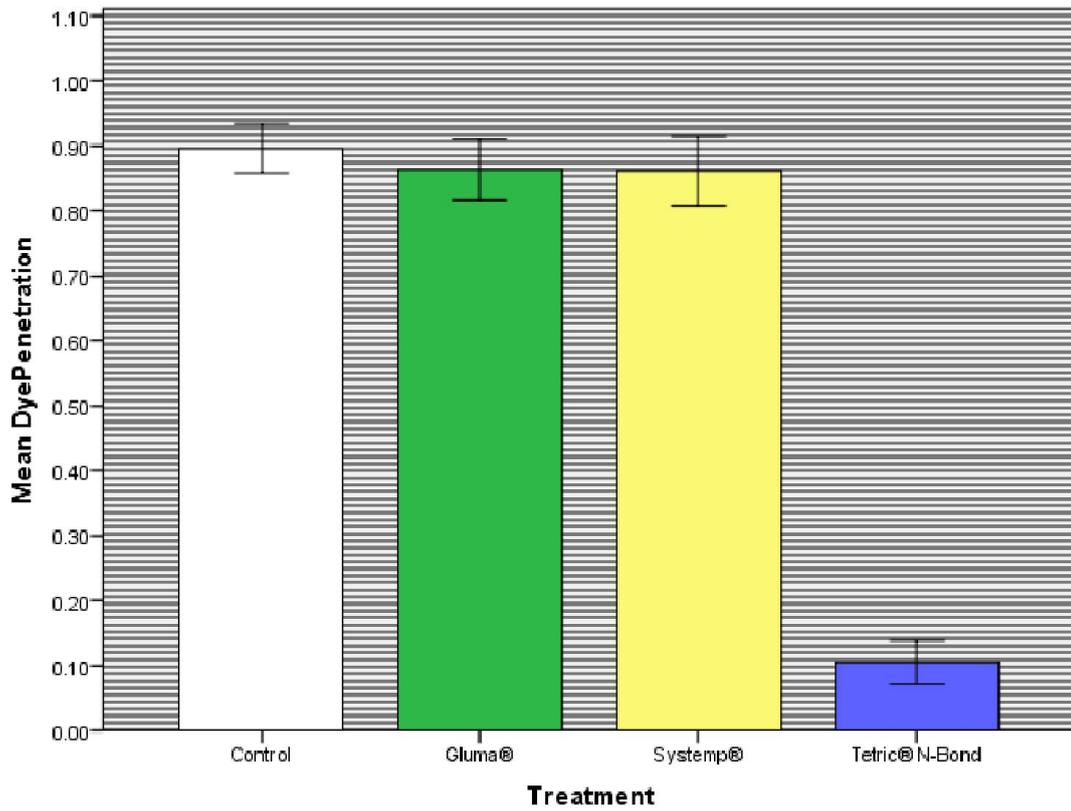


Figure 1. Microleakage mean scores, and standard deviations of the 4 Groups



Figure 2. Stereomicroscopic figure: group 1 (Control group) dye leakage reaching the pulp chamber

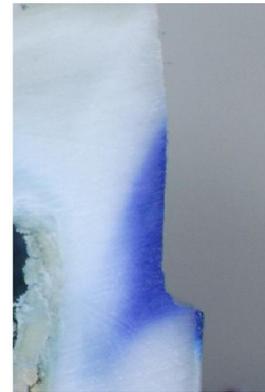


Figure 4. Stereomicroscopic figure: group 3 (Systemp® dentin desensitizer)



Figure 3. Stereomicroscopic figure: group 2 (Gluma® dentin desensitizer)

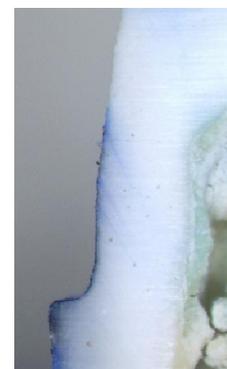


Figure 5. Stereomicroscopic figure: group 4 (Tetric® N-Bond Self-Etch dentin adhesive)

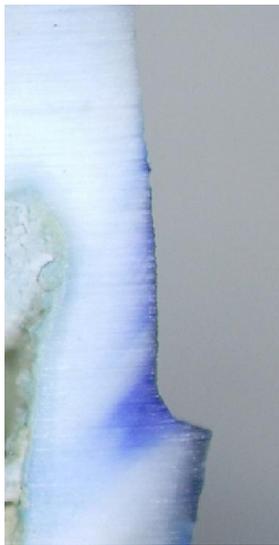


Figure 6. Stereomicroscopic figure: group 4 (Tetric® N-Bond Self-Etch dentin adhesive) Dye penetration around the internal line angles

The results demonstrated that the dentin treatments were successful in protecting exposed dentin from dye penetration. Thus, the null hypothesis was rejected because a significant difference was observed in the reduction in dye penetration between the one step self-etching adhesive and glutaraldehyde-containing desensitizers; Tetric N-Bond Self-Etch had the lowest score of mean dye penetration followed by Gluma and Systemp. The highest scores of dye penetration were observed in the negative control group without any dentin treatments.

The control group had consistently displayed dye were easily able to penetrate most of dentin wall thickness through dentinal tubules reaching the pulp chamber. This indicates high dentin permeability. Consequently, this finding supports the importance of sealing or occluding the dentinal tubules after tooth preparation for full-coverage indirect restoration. Both Gluma® and Systemp® dentin desensitizers used in this study could reduce dye penetration to some extent versus the control group. Systemp® had slightly better performance versus Gluma®, which may be explained by slight difference in their composition. Systemp® contains polyethylene glycol dimethacrylate (PEG-DMA) that also triggers the precipitation of plasma proteins in the dentinal tubules (according to manufacturer). This synergizes the effect of glutaraldehyde, and thus Systemp® precipitated more protein to block or reduce the diameter of the dentinal tubules than Gluma® which only contains glutaraldehyde for protein precipitation.

The ability of both desensitizers to reduce permeability was marginally significant versus the control group. This is inconsistent with previous clinical reports that Gluma® and Systemp® were effective in reducing pain from dentine hypersensitivity (Stewardson *et al.*, 2004; Mehta *et al.*, 2014; Arrais *et al.*, 2004). This may be because glutaraldehyde is a cross-linking reagent capable of bonding to amine groups of proteins to form highly cross-linked and insoluble protein aggregates that block or reduce the diameter of dentinal tubules (Schüpbach *et al.*, 1997). Thus, sufficient plasma proteins are

needed to ensure optimal sealing of the tubules by glutaraldehyde-containing desensitizers.

In clinical dentin hypersensitivity studies on vital teeth where plasma proteins are available naturally inside dentinal tubules fluid, and tooth preparation will evoke an inflammatory response in the pulp that leads to increased intra-pulpal pressure and interstitial content of plasma proteins (Knutsson *et al.*, 1994). Thus, more plasma proteins will be released from the pulp to the exposed ends of the dentinal tubules. This provides enough protein to react with glutaraldehyde to completely seal the dentinal tubules and reduce the dentin permeability and consequently eliminate the patients' dentin hypersensitivity. On the other hand, the current *in vitro* study was done on extracted non-vital teeth and there were relatively low protein concentrations to react with glutaraldehyde resulting in partially occluded dentinal tubules similar to scanning electron microscopic study conducted by Joshi *et al.* (2013). This explanation is confirmed by studies that use a bovine albumin pre-treatment before applying glutaraldehyde-containing desensitizers (Ishihata *et al.*, 2012; Schüpbach *et al.*, 1997). These studies showed that the agents were effective in significantly reducing dentin permeability. Therefore, the source of the protein plays a key role in reducing dentin permeability when using glutaraldehyde-containing desensitizers.

On this basis, two clinical concerns will arise when using glutaraldehyde-containing desensitizers. First, the use of anesthesia with vasoconstrictor that is essential to most restorative procedures reduces pulpal blood flow by 73% for at least 1 hour (Ahn *et al.*, 1998) this will compromise the ability of the pulp to provide enough plasma protein to react with glutaraldehyde. This reduces its efficacy by delaying dentinal tubules occlusion, this explain the longer time needed by glutaraldehyde-containing desensitizers in relieving sensitivity verses other desensitizing agents (Gupta *et al.*, 2013). Moreover glutaraldehyde is water-soluble and can be washed away easily. Thus, it may not stay in place until the return of normal pulpal blood flow with adequate plasma proteins.

Second, in endodontically treated teeth, these agents may not work properly due to a lack of sufficient protein inside dentinal tubules to react with glutaraldehyde for complete sealing of the dentinal tubules. This leads to unsealed dentinal tubules easily penetrated by bacteria and their byproducts. Indeed, several studies have shown that bacteria can colonize inside dentinal tubules to cause margin discolorations and secondary decay in endodontically treated teeth (Nagaoka *et al.*, 1995; Love and Jenkinson *et al.*, 2002).

The most satisfactory results for reduction in dentin permeability were obtained with one-step self-etching. This is consistent with a clinical hypersensitivity study conducted by Yu *et al.* (2010) who reported that one-step self-etching adhesives could significantly relieve dentin hypersensitivity immediately and over the course of a month after treatment. This was also consistent with *in vitro* dentin permeability studies conducted by Sahin *et al.* (2012) using a fluid-transport model. They showed the better performance of one-step self-etch adhesives over Gluma. Moreover the dye penetration

study conducted by Fu *et al.* (2007) also demonstrated the ability of a one-step self-etch adhesive to significantly reduce dye penetration versus the control group. The performance of this adhesive was better than the Gluma desensitizer. On the other hand, these results were inconsistent with Grégoire *et al.* (2003) who reported that self-etching adhesives reduce dentin permeability less than conventional etch-and-rinse adhesives. This is because the adhesive used in that study was a strong self-etching adhesive with a pH near 0.8. This completely removed the smear layer and left the dentinal tubules and dentinal fluids in direct contact with the adhesive. This led to poor resin penetration and hybridization of the dentin layer.

It is important to highlight that self-etch dental adhesives are a complex mixture of components including acidic adhesive monomers, cross-linking monomer, and dissolved hydrophilic monomers in addition to the solvent, filler, photo-initiator and stabilizers. Their behavior (sealing abilities, bond strength, etc.) depends on the individual composition of dentin adhesives as well as the application environment. It cannot be generalized to categories/generations. Indeed, most dental literature demonstrated that the one-step self-etch adhesive—regardless of their composition—offers a permeable membrane that permits the continuous transudation of dentinal fluid and do not provide a hermetic seal in vital deep dentine (Tay *et al.*, 2004). However, the results of this study and other studies show the ability of these agents to significantly reduce dentin permeability (Fu *et al.*, 2007; Yu *et al.*, 2010; Sahin *et al.*, 2012).

The adhesive system used here (Tetric N-Bond) is water-based. Water is the only solvent used with no co-solvent. It is a moderate self-etching adhesive with pH of 1.5. It is composed of hydrolytically stable acidic monomers and cross-linking monomer in an aqueous condition in addition to nano silica fillers. These monomers were formulated by substitution of instable ester bonds by a more stable amide bond (Moszner *et al.*, 2006). The behavior of this adhesive system was evident in the stereomicroscope photographs (Figure 6), which show limited dye penetration restricted in most of the treated dentin to the outer adhesive layer except for small areas around the internal line angle of the preparation where the dye can penetrate deeper into the dentinal tubules. This behavior can be explained by the presence of an acidic monomer and hydrophilic wetting monomer in adhesive composition. This makes it more hydrophilic and susceptible to increased water sorption (Tay and Pashley, 2003)—this explains diffusion of the dye through absorption and adsorption along with water uptake. Fortunately, the dye cannot penetrate deeper inside the dentin toward the pulp, which may be because of the moderate acidity of this adhesive which modified the smear layer keeping the smear plugs that are responsible for blockage of dentinal tubules and slowing of the fluid diffusion (Tay and Pashley, 2001). Moreover the presence of the nano filler in between the monomer network and the formation of acid resistance layer limit the diffusion to nano-scale particles (Nikaido *et al.*, 2009). This allows travel of ions and water while simultaneously sealing out large dye molecules. Deeper dye penetration around the line angles indicates that these angles are not covered properly with the adhesive to form a proper hybrid layer despite the adhesive being brushed on the

dentine for 30 s according to the manufacturer's instructions to ensure spreading of agent across the entire dentin surface. This may be because water is the only solvent and water is a poor solvent for organic compounds (such as monomers), which are usually rather hydrophobic (Sayed *et al.*, 2002). It evaporates slowly due to low vapor pressure and thus using water alone in low concentration makes this agent have a high viscosity with poor initial contact angle to dentin. (Grégoire *et al.*, 2011) This results in a longer application time and careful air-drying until the fluid film has disappeared in order to completely wet the dentin surface especially at line and point angles, and to insure complete evaporation of the water.

Self-etch dentin adhesives used in this study satisfy some of the ideal desensitizer agent characteristics described by Grossman (1935) including an initial "simple" application because it is only a one-step application. Second, it is nonirritating to the pulp and soft tissue and is relatively painless. This is because no strong acids are used that might irritate the pulp and periodontal tissue. It is water based and will not causing tooth desiccation. On the contrary it is a rewetting agent, and the monomer cannot penetrate deep to reach the pulp as the smear layer only is modified and not completely removed. Third, it acts quickly—the material is polymerized immediately by light curing. In addition, single-bottle self-etch adhesive seems to reduce dentin permeability for 3 months as reported by Delannée *et al.* (2013). This is more than enough time for tooth preparation to final cementing for good sealing and definitive restoration. Moreover, self-etch dentin adhesives have antimicrobial properties (Feuerstein *et al.*, 2007; Kim *et al.*, 2014) and can be used effectively and quickly in root canal-treated teeth (Assouline *et al.*, 2001). They are not affected by reduction in pulpal blood flow. On the contrary, reduction in the intra-pulpal pressure enhances the monomer penetration in deep dentin and formation of a thicker hybrid layer (Hashimoto *et al.*, 2000). These characteristics of self-etch dentin adhesive make it an effective, robust and attractive therapy for reduction dentin permeability and dentinal hypersensitivity.

Dentin permeability is a complex process affected by many factors including the area exposed, the structure and chemistry of the involved dentin, the thickness of the remaining dentin, the intra-pulpal pressure exerted on the process, and particle size (Mjör, 2009). Although numerous efforts have been made to address these factors, it is important to consider the limitation of *in vitro* studies. Dentin treatment in non-vital teeth in laboratory environment is not equivalent to dealing with vital dentin in the patient's mouth. The presence of positive intra-pulpal pressure may affect the proper diffusion of monomer and hybridization, and continuous outflow of plasma inside dentinal tubules and high intraoral humidity may prevent complete evaporation of water solvent from the adhesive. Even if the complete evaporation of water was successful, it will be replaced rapidly by water diffusion back from the vital bonded dentin and lead to water blisters at the dentin adhesive interface, dilution of monomer, and phase separation results in an over-wet phenomenon that leads to poor adaptation and spread of monomers in the dentine to form an inadequate hybrid layer. Thus, further clinical studies are needed to gain more insight into the clinical performance and sealing abilities of these agents.

Conclusion

Within the limitations associated with this in vitro study, self-etch dentin adhesive showed significant less dye penetration than two glutaraldehyde-containing products and the no treatment group (negative control group).

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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