Original Article

Effect of a Desensitizing Varnish on Microleakage of Two Self-Etch Adhesives

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Abstract

Objectives: The aim of this in-vitro experimental study was to assess the effect of application of a desensitizing varnish on the enamel and dentin marginal seal.

Materials and Methods: Seventy-two freshly extracted, intact human premolar teeth were divided into four groups (n=18). Class V cavities (3mm in length, 2mm in width and 2mm in depth) were prepared on the buccal surface of each tooth. The following sealing materials were applied in the four groups: One-step Clearfil S3 Bond (S3) self-etch adhesive, two-step Clearfil SE Bond (SE) self-etch adhesive, S3 Bond+ VivaSens desensitizing varnish (VS+S3) and Clearfil SE Bond + VivaSens (VS+SE). The cavities on the teeth were then incrementally filled with Z350 light-cure composite. The teeth were stored in distilled water for 24 hours at 37°C, and were then thermocycled for 1000 cycles. Then, all the specimens were prepared for dye penetration test and were immersed in 2% basic fuchsin dye and incubated at 37°C for 24 hours. The teeth were then sectioned buccolingually along the center of restorations with a diamond disk. Microleakage at the tooth-restoration interface was assessed in the enamel and dentin margins blindly using dye penetration under a stereomicroscope at ×20 magnification.

Results: There was significantly greater leakage at the enamel and dentin margins in group VS+SE than in group SE; also, these values were higher in group VS+S3 than in S3.

Conclusion: Combined application of desensitizing varnish and self-etch adhesives seems to increase microleakage in composite restorations. Thus, its application is not suggested.

Keywords: Dental Leakage; Adhesives; Dentin Sensitivity

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INTRODUCTION

Dentin hypersensitivity is characterized by short-lasting, sharp pain. This pain is the response of exposed dentin to stimuli [1]. Dentin sensitivity exacerbates when no enamel exists or when the root surface is naked. Subsequent to these two conditions, clinical problems may occur due to dentin exposure. Dentin exposure has a close association with severe dentin sensitivity. Tooth sensitivity can be a result of periodontal treatment, or inappropriate brushing habits causing gingival recession [2]. It can be treated with invasive methods including periodontal surgery, pulpectomy and laser therapy, or with non-invasive treatments including the application of dentin bonding agents and use of desensitizing dentifrices and topical agents.

It seems that non-invasive treatments can be considered as the first treatment choice since they are simple, cheap, and effective for most patients [3].

Desensitizing agents include fluoride, calcium oxalate, potassium nitrate and calcium phosphate, which block the dentinal tubule openings and consequently decrease dentin permeability [4].

Composite restorations are sometimes indicated after dentin sensitivity treatments. However, the effect of desensitizers on the restoration-adhesive bond strength is unclear. Pashley et al. reported that after the application of desensitizers, the dentin surface was no longer suitable for bonding [5]. On the other hand, self-etch adhesives are increasingly used. diffusion and provides chemical bond between adhesive and dentin substrate [6].

The other point is that use of one-step self-etch systems may lead to some problems since the composition of hydrophilic and hydrophobic components in these systems is in such a way that they show significant hydrophilic activity. Thus, they absorb water from the dental tubules via the phenomenon of osmosis.

Water absorption increases solubility, generation of hydrolytic degradation products and nanoleakage. Subsequently, bond strength decreases [7].

It has also been observed that increase in permeability of hydrophilic adhesive layer depends on the hydration of underlying dentin [8].

Table 1. Material characteristics

Material	Composition	Application procedure
Clearfil S3 Bond (Kuraray Noritake Dental Inc., Sakazu, Okayama, Japan)	10-Methacryloyloxydecyl dihydrogen phosphate (MDP), Bis-phenol A diglycidyl methacrylate (Bis-GMA), 2-Hydroxy-ethylmethacrylate (HEMA), Hydrophobic dimethacrylate, dl-Camphorquinone, Ethyl alcohol, water, silanized colloidal silica	Apply BOND to the entire cavity wall with a disposable brush tip. Leave it in place for 20 seconds. After conditioning the tooth surface for 20s, dry the entire adherent surface sufficiently by blowing high-pressure air for more than 5s.
Clearfil SE Bond (Kuraray Noritake Dental Inc., Sakazu, Okayama, Japan)	10-Methacryloyloxydecyl dihydrogen phosphate(MDP), Bis-phenol A diglycidyl methacrylate (Bis-GMA), 2-Hydro-xyethylmethacrylate (HEMA), Hydro-phobic dimethacrylate, dl Campho-rquinone, N, N-Diethanol-p-toluidine, colloidal silica	Apply PRIMER to the entire cavity wall with a disposable brush. Leave in place for 20 s. After conditioning the tooth surface for 20s, evaporate the volatile ingredients with a mild oil-free air steam. Apply Bond and distribute evenly with mild airflow. Light cure for 10s.
VivaSens (Ivoclar Vivadent AG, Schaan, Liechtenstein)	Varnish (ethanol, water and hydroxy propyl cellulose) containing potassium fluoride, polyethylene glycol dimeth-acrylate, and other methacrylates.	Gently rub liquid into tooth for at least 10s, avoiding contact with gingiva. Evenly disperse the liquid and dry by gently blowing air on the treated surfaces for 10s.
Filtek Z-350 (3M ESPE Dental Products, St. Paul, MN, USA)	Bis-GMA, UDMA,TEGDMA, Ethyl methacrylate, inorganic fillers	Two oblique increments Were applied into the cavity and cured

Bis-GMA=bis-phenol-A glycidyl methacrylate,
HEMA=2-hydroxyethyl methacry

A highly significant correlation was found between the permeability of adhesive and water drops on the underlying dentin surface with water content of the moist underlying dentin surface under pressure [9]. Leakage tests are used to assess the marginal seal. Observation of dye penetration at the bonding surface can also show leakage [10,11]. A few studies have investigated the effect of desensitizing agents on self-etch adhesive systems [2,4,5]. Thus, the purpose of this study was to assess the effect of application of a desensitizer on the enamel/dentin marginal seal.

MATERIALS AND METHODS

Seventy-two recently extracted sound human premolars without restorations or cracks were selected. The teeth were stored in saline for one month. The teeth were washed with water, and residual tissue and debris were removed by a scaler. Then, the teeth surfaces were cleaned using pumice paste and rotary instrument. The teeth were stored in 0.5% chloramine T solution at 4°C for one week for the purpose of disinfection. Class V cavities (3mm in length, 2mm in width and 2mm in depth) were prepared on the buccal surface of each tooth with occlusal margins 1mm above the cementoenamel junction and gingival margins 1mm below it, using a straight diamond bur (#878d2, Teezkavan, Tehran, Iran) in a high speed handpiece under constant air-water spray. After five preparations, the diamond bur was replaced with a new one. The teeth were stored in distilled water during the experiment to prevent dehydration.

The teeth were then randomly divided into four equal groups, (n=18).

Clearfil S3 Bond one-step self-etch adhesive (Kuraray, Osaka, Japan) was used in group 1(S3) according to the manufacturer's instructions. In group 2 (SE), Clearfil SE Bond (Kuraray, Osaka, Japan) two-step self-etch adhesive was used according to the manufacturer's instructions. In group 3 (VS+S3), VivaSens (Ivoclar Vivadent AG, Schaan, Liechtenstein) was applied on the cavity walls for 10 seconds and was then dried using air spray for 10 seconds. After that, S3 Bond self-etch adhesive was applied. In group 4 (VS+SE), VivaSens and then Clearfil SE Bond were applied as explained above. Table 1 shows the composition of materials used in the present study and their manufacturers' instructions.

The cavities were filled with Z350 light-cure composite (3M ESPE, St. Paul, MN, USA) in two oblique increments. Each increment was light cured using an LED light curing unit (Valo; Ultradent Products Inc., South Jordan, UT, USA) with a light intensity of 1000mW/cm^2 for 20 seconds. The restorations were polished and finished using Opti-Disc (OptiDisc, Kerr, Orange, USA). The specimens were stored in distilled water at 37°C for 24 hours, and were then thermocycled (TC-300, Vafaei Industrial, Tehran, Iran) for 1,000 cycles between 5°C-55°C, with a dwell time of 30 seconds. The root apices were sealed with sticky wax.

Then, all surfaces of each tooth were covered with two layers of nail varnish except for one-millimeter margin around the restoration. Then, the specimens were immersed in 2% basic fuchsin (Ranbaxy Fine Chemicals Ltd., New Delhi, India) dye at 37°C for 24 hours. The teeth were then rinsed with water and blot-dried. The teeth were mounted in a cutting machine (Mecatome, T201A, Persi, Grenoble, France) using transparent polyester acrylic resin. The teeth were then sectioned buccolingually along the center of restorations, using a 0.3 mm thick two-sided diamond disk under water.

Table 2. The pH values

Material	pH value
Clearfil S3 Bond	2.7
Clearfil SE Bond	1.9

Two blind observers examined the enamel and dentin margins of the two sections of each tooth under a stereomicroscope (SMZ800, Nikon, Tokyo, Japan) at ×20 magnification according to the following scale: 0: No leakage; 1: Leakage up to one-third of the length of the cavity wall; 2: Leakage up to two-thirds of the length of the cavity wall, not including the axial wall and 3: Leakage along the axial wall [10].

Measurement of pH:

A digital pH meter (WTW523, Wissenschaftlich-Technische Werkstatten GmbH, Weilheim, Germany) was used in a dark room with special red light at room temperature to measure the pH of adhesive. Ten drops of adhesive were used to measure the pH value. The pH value was recorded after 15 seconds when the device showed a constant figure (Table 2). Statistical analysis was performed using the non-parametric Kruskal-Wallis test, and post-hoc Dunn's test was applied for pairwise comparison of leakage between enamel and dentin margins in the groups. The level of statistical significance was set at $P<0.05$.

RESULTS

The amount of dye penetration and the scores of the enamel and dentin margins are presented in Tables 3 and 4.

Significant differences existed among the groups regarding microleakage at both enamel ($P=0.001$) and dentin ($P<0.001$) margins, according to the Kruskal-Wallis test. Paired comparisons by Dunn's test revealed that the differences in microleakage at the enamel margins between groups S3 and SE($P=0.01$), S3 and VS-S3 ($P=0.046$), and SE and VS-SE ($P=0.026$) were significant. Regarding micro-leakage at dentine margins, significant differences were found between groups S3 and SE ($P<0.001$), S3 and VS-S3 ($P=0.001$) and SE and VS-SE ($P<0.001$) (Table 5).

DISCUSSION

According to the results of this study, use of desensitizing varnish along with Clearfil SE Bond and S3 Bond self-etch adhesives caused a significant increase in microleakage. The findings showed significant differences between S3 and SE groups, VS+S3 and S3 groups and also VS+SE and SE groups, at both margins. Two-step Clearfil SE Bond self-etch adhesive was used in this study. This adhesive is used as the gold standard of self-etch adhesives in studies on bond to dentin [12]. One-step Clearfil S3 Bond self-etch adhesive was also used in the current study.

The manufacturer of this adhesive claims that the difference between Clearfil S3 Bond self-etch adhesive and other adhesives is due to the

Table 3. Distribution of dye leakage in the dentin margin according to the scoring system

Group	Dentin leakage score N(%)				Total
	0	1	2	3	
S3	0(0)	0(0)	2(11.1)	16(88.8)	18(100)
SE	1(5.5)	12(66.6)	5(27.7)	0(0)	18(100)
VS+S3	1(5.5)	1(5.5)	14(77.7)	2(11)	18(100)
VS+SE	0(0)	1(5.5)	9(50)	8(44.4)	18(100)
Total	2(2.7)	14(19.4)	30(41.6)	26(36.1)	72(100)

presence of MDP in its formulation, which maintains the homogeneity of S3 Bond and prevents phase separation that occurs in acetone-based all-in-one adhesives [13].

A recent study showed that S3 Bond was more resistant to biomechanical stresses compared to older two-bottle adhesives [14]. Application of SE Bond in Class V cavities results in a good retention rate (more than 90%) after two to three years in the clinical setting [15].

On the other hand, one way to decrease postoperative tooth hypersensitivity is to apply desensitizing agents [2]. Oxalate desensitizers have been previously studied. However, the effect of VivaSens desensitizing varnish containing potassium fluoride on bond strength and marginal seal is unclear [4].VivaSens decreases the dentin sensitivity through sealing the dentinal tubules. Its mechanism of action is via precipitation of proteins and calcium ions in extra-tubular fluid. It also causes co-precipitation of poly ethylene glycol dimethacrylate (PEG-DMA), which is present in its formulation [16]. From the biochemical point of view, it has been clearly understood that organic acids and solvents can be used to promote protein precipitation. If higher amounts of poly ethylene glycol are added to a protein solution such as blood plasma, the solubility of the proteins decreases, and some of the proteins start to precipitate [17]. VivaSens also contains organic acids (such as phosphoric acid methacrylate) and ethanol solvent, which induce protein precipitation in tubular liquid. The second function is that acid induces salt formation. Tubular liquid is rich in calcium ions.

Phosphoric acid methacrylate forms calcium salts with low solubility. Thus, deposits are formed in tubules. Another acidic component in the formulation of this desensitizer is methacrylate modified polyacrylic acid, which is a complex builder, and promotes the formation of more salts. Finally, superficial blocking of the tubules is achieved though application of VivaSens as a hydroxy propyl cellulose film former. This film seals the dentinal tubules transiently and blocks tubular fluid flow. Thus, it stops nerve stimulation and pain sensation [16].

According to the findings of the current study, microleakage significantly increased when VivaSens was applied on enamel margins in use of both SE and S3 bonding systems. Previously, Tay et al. [18] reported that enamel-resin bonding process is affected by formation of calcium oxalate crystals on etched enamel. These crystals can be easily removed by re-etching so that hydroxyapatite crystals in the enamel beneath the calcium oxalate crystals are well etched when calcium oxalate crystals are removed.

Table 4. Distribution of dye leakage in the enamel margin according to the scoring system

Group	Enamel leakage score N(%)				Total
	0	1	2	3	
S3	3(16.6)	12(66.6)	3(16.6)	0(0.0)	18(100)
SE	12(66.6)	6(33.3)	0(0.0)	0(0.0)	18(100)
VS+S3	11(61.1)	6(33.3)	1(5.5)	0(0.0)	18(100)
VS+SE	4(22.2)	11(61.1)	3(16.6)	0(0.0)	18(100)
Total	30(41.6)	35(48.6)	7(9.7)	0(0.0)	72(100)

Thus, penetration of resin is improved. It seems that this explanation also applies to the current study since application of calcium oxalate showed no adverse effect on the enamel [18]. It seems that a similar mechanism in the current study decreased marginal seal after the application of VivaSens on the enamel. The acidity of acidic resin monomer in these two self-etch bonding agents is not sufficient to remove the precipitations and provide a desirable bond [15].

The results of the current study showed that application of desensitizer on dentin before the application of SE and S3 bonding agents increased microleakage and decreased marginal seal. This finding is in line with that of Arisu et al. who showed a decrease in bond strength following the application of SE adhesive [19].

The most important ingredients of self-etch adhesives are resin monomers which contain cross-linkers and functional monomers. [10]. Two self-etch adhesives namely two-step Clearfil SE Bond (pH=1.9) and one-step Clearfil S3 Bond (pH=2.7) were used in this study. Findings of the current study showed significant differences between these two adhesives regarding the increase in microleakage after the application of desensitizers.

The primer and bonding agent of two-step self-etch adhesives contain a mixture of resin monomers; light-, chemical- or dual-cure initiators and other additives. Water is also present in all self-etch primers as an ionizing medium enabling the primer to perform etching [20]. The acidic primers, which contain functional and hydrophilic monomers, enable the demineralization by self-etch adhesives [21]. Their demineralization potential depends on their pH values; adhesives with pH≤1 are considered strong, and those with pH≥2 are considered mild [22]. Mild self-etch systems such as SE have a pH around 2, and cause minimal etching of the enamel. They result in formation of narrow and shallow resin tags with approximately 1µm of penetration depth [23]. The hybrid layer formed by mild self-etch systems is thinner and provides less prominent resin tags compared to strong self-etch and total etch systems. Compared to strong self-etch and etch and rinse systems, formation of resin tags is less eminent in mild self-etch systems [24]. However, high bond strength, similar to that in the etch and rinse systems, has been reported following the application of these systems [25].

One-step self-etch adhesives are a mixture of hydrophilic and hydrophobic components, which produce narrower hybrid layers compared to etch and rinse and two-step self-etch systems [10]. They are less technique-sensitive, which is an advantage [10]. However, some studies have shown that narrower hybrid layers are responsible for lower polymerization [26] and higher permeability [27,8]. They are also very hydrophilic and absorb water from dentinal tubules via the osmotic phenomenon [7]. The other point in application of self-etch adhesives is adhesion-decalcification concept. Monomers such as 4-methacryloxyethyl trimellitic acid, phenyl-p and MDP are components of acidic primers and have potential for chemical bond to calcium hydroxyapatite. According to this concept, all acids interact with calcium hydroxyapatite and form ionic bonds.

Table 5. P value among groups in the enamel and dentin

Enamel		Dentin	
Groups	**P value**	**Groups**	**P value**
SE – VS+S3	>0/999	SE – VS+S3	=0/067
SE – VS+SE	=0/026	SE – VS+SE	<0/001
SE - S3	=0/10	SE - S3	<0/001
VS+S3 – VS+SE	=0/103	VS+S3 – VS+SE	=0/549
VS+S3 – S3	=0/046	VS+S3 – S3	=0/001
VS+SE – S3	>0/999	VS+SE – S3	=0/216

The acidic monomer used in the adhesives evaluated in the current study was MDP, which seems to be one of the most effective monomers in forming chemical bonds to dentinal substrate. It can create ionic bonds to hydroxyapatite even within 36 seconds of exposure. This interaction can produce stable salts on the surface of both enamel and dentin [28]. According to the results of the current study, it seems that after the application of VivaSens on dentin, less calcium salts are available for resin monomers. Subsequently, it seems that with a decrease in chemical bonds, the microleakage increases. The other point is the difference between the acidity of the two adhesives used in the current study. Although the difference in pH between the two adhesives is about 0.8, according to the results of the current study, it is probable that the lower pH of SE caused more structural changes in surfaces compared to S3, leading to less microleakage. However, the difference in microleakage was not significant. Further studies using scanning electron microscopy are suggested to investigate this concept.

CONCLUSION

With regard to the limitations of the present study, the results showed that application of a desensitizing varnish in addition to Clearfil SE Bond and S3 Bond self-etch adhesives increased microleakage in both enamel and dentin margins in composite restorations and interfered with the function of adhesives. Thus, combined use of desensitizing varnish and adhesives is not recommended.

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