Original Article

Effect of Re-etching of Oxalate-Occluded Dentin on Microleakage of Composite Resin Restorations

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Abstract

Objectives: This study aimed to assess the effect of re-etching of desensitized dentin for five and 10 seconds on marginal microleakage of composite restorations.

Materials and Methods: Class V cavities (4x2x2mm) were prepared on the buccal surfaces of 64 third molars and randomly divided into four groups of 16. In the control group, Single Bond (SB) adhesive was applied after etching. In BB+SB group, after application of BisBlock (BB) desensitizer agent (which needs etching), SB adhesive was used. In BB+5E+SB group, re-etching of dentin was done for five seconds after application of desensitizer, and then the adhesive was applied. The process in BB+10E+SB was the same as BB+5E+SB group except for re-etching time, which was 10 seconds. The cavities were restored with composite resin. After 24 hours of storage in distilled water and 10,000 thermal cycles, all samples were subjected to dye penetration test. The teeth were sectioned buccolingually in the middle of restorations. A blind examiner observed the sections under a stereomicroscope.

Results: At the occlusal margins, no significant difference in microleakage was observed among the groups (P>0.05). Application of BB in combination with SB had no effect in comparison to the control group (P>0.05); while there were significant differences in microleakage scores between BB+10E+SB and control (P=0.002), BB+10E+SB and BB+SB (P<0.001) and BB+5E+SB and BB+SB groups (P=0.009).

Conclusions: Dentin re-etching after application of BB desensitizer increased the gingival microleakage of class V composite restorations. Application of BB desensitizer combined with SB adhesive enhanced marginal seal.

Keywords: Oxalates; Acid Etching, Dental; Composite Resins; Dental Marginal Adaptation

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INTRODUCTION

Despite the long-term success of etch and rinse (E&R) adhesives, their clinical success still faces some problems. Increased dentin permeability due to removal of smear layer interferes with infiltration of resin monomer and leads to its dilution and consequently increased sensitivity of the restored tooth [1,2].

In order to decrease the exit of dentinal fluid from the etched surface during the bonding process, Pashley et al, [3] suggested the application of oxalate desensitizer agents on the acid-etched surface prior to adhesive application. It is assumed that decreasing the calcium ions on the dentin surface leads to greater diffusion of oxalate ions into the dentinal tubules. The formed calcium oxalate crystals seal the dentinal tubules and decrease the permeability of dentin without compromising the bonding process [3]. Simultaneous usage of several formulations of desensitizer agents and different adhesives has been investigated [4-9].

Yiu et al, [7,8] showed that combination of desensitizer with E&R adhesives, which had relatively high pH and no fluoride did not affect the quality of bond. However, application of fluoride-containing E&R adhesives with low acidity interfered with the bonding procedure due to formation of spherical globules on the surface of dentin, which interfered with the hybridization process of demineralized dentin. In another study, Tay et al, [5] concluded that poor bond of E&R adhesives to dentin treated with oxalate was a result of interference caused by acid-resistant oxalate crystals. They believed that in order to achieve efficient sealing of dentinal tubules and bond to dentin, calcium removal by acid should take place before application of desensitizer agent. Moreover, tooth hyper-sensitivity following composite restorations results from increased microleakage or marginal permeability to fluids, bacteria and their enzymes. This is due to the formation of micro-gaps at the restoration-dentin interface resulting from adhesive failure [10]. Shafiei et al, [11] observed that application of oxalate after acid etching may increase microleakage of dentin margins when applying low pH (E&R) adhesives, but it had no effect on microleakage of adhesives with neutral pH. Another study showed that application of BisBlock (BB) resulted in higher microleakage at the dentin margins compared to other surface pretreatments [12].

In addition, solubility of oxalate is affected by pH since the anions are the basis of weak acids [8].

Yousry [13] suggested that some of the oxalate desensitizing agents might need an extra etching process in order for the substrate to have better bonding ability. It was shown that after re-etching, oxalate crystals in dentinal tubules were fewer and smaller in size as revealed by scanning electron microscopic examination [13]. However, this concept has not been further investigated to decrease microleakage of dentinal cavity walls.

The aim of this study was to evaluate the effect of different dentin re-etching time periods (five or 10 seconds) following oxalate treatment on microleakage of composite restorations.

The null hypothesis was that re-etching of dentin treated with oxalate desensitizer agent for five or 10 seconds would not decrease the microleakage at the dentinal margin of composite restorations.

MATERIALS AND METHODS

Sixty-four newly extracted, caries-free, sound human third molars without any cracks were collected and stored in normal saline for one month. The teeth were stored for one week in 0.5% chloramine T solution at 4°C for disinfection. Standard class V cavities (4mm long, 2mm high, 2mm deep) 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 bar (# 878/2d; Teeskavan, Tehran, Iran) with a high speed headpiece under constant air-water spray. After five preparations, the diamond bur was replaced with a new one. Enamel cavosurface line angles were not beveled. Adper Single Bond 2 (3M ESPE, St Paul, MN, USA) was used as an E&R adhesive and BB (Bisco Inc., Schaumburg, IL, USA) as a desensitizer agent according to their manufacturers' instructions (Table 1).

Measurement of pH: A digital pH meter (WTW, 523, Wissenschaftlich-TechnischeWerkstätten GmbH, Wilhelm, Germany) was used in a dark room slightly lit with a special red light at room temperature to measure the pH of adhesive [8]. Ten drops of adhesive were used for pH measurement. Water-free adhesives that dissolve in polar solvents do not usually dissociate to ionic species. Thus, a solution of adhesive in 70% ethanol and 30% distilled water was prepared in a clean glass vial. The pH was recorded after 15 seconds when the device showed a constant figure. The pH of Adper Single Bond 2 was 3.6 and that of Scotchbond Etchant was 0.1.

The teeth were randomly assigned to four groups of 16 teeth each:

Table 1: Materials used in this study

Material	Composition	Application procedure
Adper Single bond 2 (3M ESPE Dental Products, St. Paul, MN, USA)	Bis-GMA, HEMA, dimethacrylates, ethanol, water, photoinitiator, methacrylate functional copolymers of polyacrylic and polyitaconic acid, silica-nano fillers	Applied in 2-3 coats with gentle agitation for 15 seconds, gently air thinned for 5 seconds and light cured for 10 seconds
BisBlock (BISCO, Inc., Schaumburg, IL, USA)	Oxalic Acid<%5/0800011529	Etched for 15 seconds, rinsed and gently air-dried for two to three seconds, BisBlock applied and dwelled for 30 seconds, rinsed, re-etched enamel for 15 seconds, rinsed, adhesive applied
Scotchbond Etchant (3M ESPE Dental Products, St. Paul, MN, USA)	34% phosphoric acid	15 seconds of etching+15 seconds of rinsing
Filtek Z-350 (3M ESPE Dental Products, St. Paul, MN, USA)	Bis-GMA, UDMA, TEGDMA, Ethyl-methacrylate, inorganic fillers	Two oblique increments applied in cavity and cured

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

SB group (control): The cavities were etched with 34% phosphoric acid (3M ESPE Dental Products, St. Paul, MN, USA) for 15 seconds, rinsed for 15 seconds, and blot-dried. Then the adhesive was applied.

SB+BB group: Etching was done as for the control group. After drying for three seconds, BB desensitizer agent and the adhesive were applied (according to the manufacturers' instructions).

BB+5E+SB group: Etching was done as for the control group. After drying for three seconds, BB desensitizer agent was applied. Re-etching of the dentin for five seconds and rinsing for 15 seconds were done; the teeth were blot-dried, and adhesive was applied.

BB+10E+SB group: All the procedures were done the same as in previous group, except for the re-etching time of the dentin, which was 10 seconds in this group.

The cavities were filled with Z350 composite (3M ESPE, St. Paul, MN, USA) applied in two oblique increments (one millimeter each). Each composite layer was cured separately using LED light curing unit (Valo; Ultradent, South Jordan, UT, USA) with 1000mW/cm^2 light intensity for 20 seconds. The restorations were finished using carbide finishing burs and Opti-Disk (Kerr Corporation, Orange, CA, USA). The teeth were incubated for 24 hours at 37°C in distilled water and were then thermocycled (TC-300; Vafaei industrial, Tehran, Iran) for 10,000 cycles between 5-55°C, with a dwell time of 30 seconds and transfer time of 15 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 the restoration and 1mm margin around it. The specimens were immersed in 2% basic fuchsine (Carlo Erba, Millan, Italy) dye solution at 37°C for 24 hours separately. The teeth were completely rinsed with water and blot-dried. The teeth were then sectioned (0.3mm) buccolingually approximately through the center of restorations (T201 A, Mecatome, Grenoble, France).

Dye penetration was assessed blindly using a stereomicroscope at ×20 magnification (SMZ800; Nikon, Tokyo, Japan) according to the following scale:

0: No dye penetration; 1: Dye penetration up to one-third of the length of the cavity wall; 2: Dye penetration up to two-thirds of the length of the cavity wall, not including the axial wall; and 3: Dye penetration along the axial wall. The Kruskal-Wallis and Dunn tests were used for statistical analysis. The level of statistical significance was set at P<0.05.

RESULTS

The amount of dye penetration in the enamel and dentin margins is presented in Tables 2 and 3.

Table 2: Distribution of dye leakage scores at the dentin margins

Group	Dentin leakage score N(%)			
	0	1	2	3
SB (Control)	0	7(43.8)	8(50)	1(6.3)
BB+SB	0	10(62.5)	5(31.3)	1(6.3)
BB+5E+SB	0	2(12.5)	7(43.8)	7(43.8)
BB+10E+SB	0	0	6(37.5)	10(62.5)

The Kruskal-Wallis test showed no significant difference among the four groups in microleakage at the enamel margins (P=0.917). But the difference in microleakage at dentin margins was significant (P<0.001).

Table 3: Distribution of dye leakage scores at the enamel margins

Group	Enamel leakage score N(%)			
	0	1	2	3
SB (Control)	7(43.8)	9(56.3)	0	0
BB+SB	8(50)	7(43.8)	1(6.3)	0
BB+5E+SB	9(56.3)	7(43.8)	0	0
BB+10E+SB	8(50)	7(43.8)	1(6.3)	0

Pairwise comparisons by Dunn test (Table 4) revealed that the highest microleakage score belonged to BB+10E+SB and the lowest score belonged to BB+SB group. No significant difference existed between SB and BB+SB groups (P>0.999), SB and BB+5E+SB groups

Table 4: Pairwise comparisons between various groups

Groups	Control SB	BB+SB	BB+5E +SB	BB+10E +SB
SB (Control)	-	P>0.999	P=0.074	P=0.00*
BB+SB	-	-	P=0.009*	P<0.00*
BB+5E+SB	-	-	-	P>0.999
BB+10E+SB	-	-	-	-

*Statistically significant (P<0.05)

(P=0.074) or BB+5E+SB and BB+10E+SB groups (P>0.999) regarding microleakage. The microleakage in BB+5E+SB group was significantly more than that in BB+SB group (P=0.009). The microleakage in BB+10E+SB group was significantly more than that in BB+SB group (P<0.001). The microleakage in BB+10E+SB group was significantly more than that in SB group (P=0.002).

DISCUSSION

The present study assessed the marginal microleakage of oxalate desensitized dentin after re-etching. Based on the results of the present study, the group with 10 seconds of dentin re-etching showed a significant increase in microleakage compared to the control group. Thus, the null hypothesis was not accepted. The results also showed significantly more microleakage in BB+5E+SB and BB+10E+SB groups compared to BB+SB group. In the present study, dye penetration method was used because it allows gross evaluation of the quality of the interface. The significant role of hybridization in marginal leakage and bond strength has been previously reported [14]. Any interference with formation of hybrid layer has an adverse effect on marginal leakage [14]. In addition, application of BB removes calcium from the surface and allows the oxalate crystals to form deep in dentinal tubules [15]. Oxalate desensitizing agents interact with calcium ions in dentin and dentinal fluid, and form insoluble calcium oxalate ions [16]. Various forms of crystals including calcium oxalate monohydrate, dehydrate and trihydrate in hexagonal, rhomboid and quadrangular shapes are formed. It has been reported that spherical oxalate crystals are found at a pH of 8, while hexagonal ones are found at a pH of 4 [6]. Thus, precipitation of oxalate crystals in various sizes depends on the density of the active material and its acidity [17]. Yiu et al, [8] reported that oxalate desensitizing agents had no negative effect on bond quality of Single Bond (3M ESPE) and One Step (Bisco Inc.). However, they reported that adhesives with low pH and high concentration of fluoride were incompatible with oxalate agents since spherical crystals were formed [8]. These crystals blocked open tubules in cervical dentin [16]. According to the results of the current study, no significant difference was found among the four study groups regarding microleakage at the enamel margins. In dentin, however, the results were different. No significant difference in microleakage was observed between the control and BB+SB groups. This was in agreement with the results of Shafiei et al [11]. Also, BB+SB group showed the least microleakage among the study groups. Tay et al, [5] suggested to etch the dentin before application of oxalate desensitizers, because it was previously observed that the adhesive systems were unable to bond effectively to dentin treated with oxalate since calcium oxalate crystals cover the surface of the dentin and the orifice of the tubules. This acid-resistant layer can make etching ineffective and interfere with the hybrid layer [5]. Consequently, a gap is created between oxalate-treated dentin and the restoration [18].

The initial etching of dentin removes calcium ions from superficial dentin and results in formation of calcium oxalate crystals that seal the dentinal tubules in subsurface layers. Thus, this process does not interfere with hybrid layer and therefore does not affect the dentin bond strength [3,5,18]. On the other hand, De Andrade e Silva et al, [9] showed that after one-year storage period, the decrease in bond strength of SB+BB was lower than that of the control group. However, this decrease in bond strength was observed after moderate to long-term water storage; thus, it can be due to the effect of water sorption causing resin swelling and subsequently decreased inter-polymer frictional forces and weakened mechanical properties [19]. Hydrophilic resins such as Single Bond are highly susceptible to water sorption, and their internal strength is weakened by plasticization and hydrolytic degeneration [20]. It has been proposed that presence of calcium oxalate relatively blocks movement of fluid in dentinal tubules. Reduction in water content induced by osmotic phenomenon during bonding facilitates evaporation of the solvent and decreases the amount of water locked in the adhesive. It also decreases the hydrophilic degradation of adhesive induced by water sorption over time [3,19,21]. This may explain the results of the present study especially because this result was obtained after 10,000 thermal cycles. Moreover, Yiu et al, [8] reported that the solubility of calcium oxalate depended on pH, since the anion is the conjugate of a weak acid. According to Le Châtelier's principle, the higher the exposure of calcium oxalate to H_3O^+, the greater the decomposition of crystals to oxalate and calcium ions in order to maintain this balance [22]. On the other hand, Yousry [13] observed smaller and fewer oxalate crystals in dentinal tubules under a scanning electron microscope after re-etching of dentin for 15 seconds due to increased solubility of calcium oxalate. They showed that oxalate crystals were not removed completely from the dentin surface after re-etching of dentin for 15 seconds and the bond strength was compromised in their study. It has been proposed that these ions, especially oxalate ions are not completely removed by rinsing, and they interfere with the process of hybrid layer formation [13]. Moreover, further etching might accelerate hydrolytic degradation due to insufficient infiltration of resin at the resin-dentin bond, and probably activate matrix metalloproteinases [21,23]. According to the results of the present study, while the microleakage in BB+10E+SB group was significantly more than that in the control group, this was not the case in BB+5E+SB group. In addition, according to observations by Yousry [13], this might result from longer etching and greater surface roughness in BB+10E+SB group compared to BB+5E+SB group. It seems that successful combination of desensitizers and adhesives depends on the type of E&R adhesive used [5,9,11]. According to the results, it seems that use of Single Bond and BB simultaneously does not affect the quality of adhesive-dentin bond. It even led to better results than the control group after 10,000 thermal cycles in our study. On the contrary, re-etching of dentin treated with oxalate cannot be suggested either for five or 10 seconds. Further studies on this topic are encouraged especially on bond strength, scanning electron microscopic examination of the surface, and long-term results.

CONCLUSION

Under the conditions of this study, re-etching of dentin after application of BB oxalate desensitizer agent for five or 10 seconds increased microleakage at the dentinal margins of composite restorations.

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