

## Effect of Chlorhexidine Application Protocol on Durability of Marginal Seal of Class V Restorations

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### Abstract

**Objectives:** This study aimed to evaluate the effect of chlorhexidine (CHX) application protocol on durability of marginal seal of class V composite restorations.

**Materials and Methods:** Class V cavities (4×2×1.5mm) were prepared in the buccal surfaces of 160 human third molars. The teeth were randomly divided into five groups (n=32) of (G1) CHX+rinse+etching, (G2) CHX+etching, (G3) etching+CHX+rinse, (G4) etching+CHX, and (G5) etching alone as the control group. Adper Single Bond 2 was applied as an etch and rinse (E & R) bonding system. Cavities were filled with Z350 composite. Half of the teeth in each group were stored in distilled water for 24 hours and the other half were thermocycled (10,000 cycles, 5-55°C). For dye penetration test, the teeth were sectioned buccolingually and assessed at the enamel and dentin margins under a stereomicroscope at ×20 magnification. The Kruskal-Wallis, Dunn and Mann-Whitney tests were used for statistical analyses.

**Results:** There was no significant difference in immediate microleakage among the groups at the enamel and dentin margins (P= 0.894). After thermocycling, there was significantly less microleakage in G4 than the control group at dentin margin. There were significant differences in microleakage between G4 and G1 (P=0.002), G4 and G2 (P=0.001) and G4 and G5 (P= 0.001) at dentin margin.

**Conclusions:** Application of CHX after etching without rinsing is effective to decrease microleakage. However, it has no effect if applied before etching in use of this particular type of E & R adhesive after thermocycling.

**Keywords:** Dental Leakage; Chlorhexidine; Composite Resins

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### INTRODUCTION

Microleakage is defined as penetration of fluids, bacteria, molecules and ions through the interface of restorative material and cavity wall, which is not clinically detectable [1]. This phenomenon can cause marginal staining of restorations, postoperative tooth hypersensitivity, secondary caries, pulpal pathology, or complete loss of restoration [1]. In spite of chemical and technical advances in adhesive systems, obtaining an adequate gingival seal in dentin is still a major problem. In order to evaluate the marginal seal and the performance of hybrid layer, the specimens are submitted to leakage tests. Thus, degree of dye penetration through the bonding interface can reveal the

adaptation of restorative material to cavity walls [2].

An important cause of resin-dentin degradation is incorporation of ionic or polar monomers in single step etch-and-rinse (E & R) adhesive systems, which induce water sorption and consequent hydrolysis [3]. Another important reason of resin-dentin degradation is the presence of matrix metalloproteinases (MMPs), which can degrade the unprotected exposed collagen fibrils [4]. The MMPs are zinc and calcium-dependent endopeptidases present in the saliva, dentinal fluids and bacterial products [5,6]. They are capable of degrading the organic matrix of demineralized dentin [7]. It should be noted that application of phosphoric acid with a low pH for

dentin etching may partially denature the MMPs. On the other hand, mild acids such as those incorporated in simplified E & R systems can activate MMPs [8].

Even if a perfect seal is achieved with a restoration, residual bacteria can still proliferate and cause pulpal irritation and deteriorate the problems related to microleakage overtime [9].

To solve this problem, cavity disinfectant solutions have been introduced [10]. Some studies have recommended the application of chlorhexidine (CHX) before bonding procedures because of its antimicrobial effects [11,12]. Pashley et al, [5] reported that application of CHX to acid-etched dentin may be a preventive strategy against degradation of collagen fibrils. As a result, in addition to its known disinfectant effect, CHX acts as a MMP inhibitor [5]. Also, CHX can prevent or reduce the degradation of exposed collagen fibrils present in imperfect hybrid layers. Therefore, the stability of the hybrid layer is preserved over time [13]. Moreover, CHX application can be a more practical method than other techniques for rehydrating the dried demineralized dentin in order to preserve the necessitous humidity to preserve expansion of collagen network [14]. In addition, there is controversy in the literature about the effect of CHX on bonding strength or microleakage of restorations [6,7,15-17]. In fact, to our knowledge, little information exists about sealing ability and durability of E & R adhesives in combination with CHX. Thus, the present in-vitro study aimed to assess the marginal sealing ability of CHX with different application protocols in class V restorations. Our hypotheses were: 1. CHX would have no adverse effect on microleakage of class V cavities. 2. CHX application methods prior to bonding procedure (before or after acid etching) would not affect microleakage after thermocycling.

## MATERIALS AND METHODS

One hundred and sixty caries-free, intact human

third molars were used in this study. Tissue residues and calculus were removed and the crowns were cleaned with a rotary dental prophylaxis instrument. The teeth were stored in 1% chloramine T solution at 4°C for one week before use. Standard class V cavities (4mm long, 2mm high, 1.5mm deep) were prepared in the buccal surface of each tooth, with occlusal margins 1mm above the cemento-enamel junction and gingival margins 1mm below it, using a straight diamond bur (78 #8, d2; Teeskavan, 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 specimens were randomly assigned to five groups of 32 teeth each, corresponding to each application protocol.

**Group 1 (CHX+rinse+etching):** In this group, 1mL of 2% CHX solution (Consepsis, Ultradent Product Inc., South Jordan, UT, USA) was applied for 35 seconds in cavities, rinsed with water spray for 20 seconds, etched with 37% phosphoric acid (3M ESPE, St. Paul, MN, USA) for 15 seconds, rinsed with water spray for 15 seconds and gently air dried for 10 seconds. Two layers of Adper Single Bond 2 adhesive (3M ESPE, St. Paul, MN, USA) were then applied.

**Group 2 (CHX+etching):** Consepsis CHX solution was applied before acid etching as in group 1, with an exception that it was just air dried with no rinse off.

**Group 3 (etching+CHX+rinse):** Consepsis CHX solution was applied after acid etching for 35 seconds, rinsed for 20 seconds and carefully blot-dried with a sterile microbrush. Acid etching and bonding procedures were performed as previously described.

**Group 4 (etching+CHX):** Consepsis CHX solution was applied after acid etching as in group 3, with an exception that it was just air-dried without rinsing.

**Group 5 (control):** The cavities were only treated with 37% phosphoric acid (3M ESPE, St. Paul, MN, USA) for 15 seconds.

For all the cavities, the bonding and filling protocols were performed using Adper Single Bond 2 (3M ESPE, St Paul, MN, USA) two-step E & R adhesive and Filtek Z350 (3M ESPE, St. Paul, MN, USA) nanohybrid composite in two oblique increments. Curing was performed using Valo light curing unit (Ultradent Product Inc., South Jordan, UT, USA) with 1000mW/cm<sup>2</sup> light intensity for 20 seconds. All the materials were used according to the manufacturers' instructions.

Table 1 shows the composition of materials used in the present study and manufacturers' instructions. Following the pretreatment procedure in each group and filling of cavities, the restorations were finished using carbide finishing burs (#448L,012; Ultradent Product Inc., South Jordan, UT, USA) and Kerr discs (Opti Disc; Kerr, Orange, CA, USA).

Half of the specimens in each group were randomly selected and stored in distilled water at 37°C for 24 hours until microleakage testing. The remaining half were thermocycled for 10,000 cycles between 5°C to 55°C, with a dwell time of 30 seconds and transfer time of 15 seconds. After storage or thermocycling, the specimens were blot-dried and the root apices were sealed with modeling wax. The specimens were immersed in 2% basic fuchsin dye at 37°C for 24 hours separately.

After dye storage, the teeth were rinsed with water in order to remove excess dye. The teeth were then sectioned buccolingually through the center of restorations with a diamond disc (Leitz1600, Leica Instruments GmbH, Koln, Germany) under water. Dye penetration was assessed using a stereomicroscope (SMZ800, Nikon, Tokyo, Japan) at ×20 magnification. Both gingival (dentin) and occlusal (enamel) margins were evaluated by two blind examiners using the following scale:

0: No leakage;

1: Leakage up to one-third of depth of the cavity wall;

2: Leakage up to two-thirds of depth of the cavity wall, not including the axial wall; and

3: Leakage along the axial wall [1].

**Statistical analysis:** The Kruskal-Wallis test, Dunn test and Mann-Whitney test were used for statistical analyses. The level of statistical significance was set at  $P < 0.05$ .

## RESULTS

The amount of dye penetration and the microleakage scores of the enamel and dentin margins are presented in Table 2. The Kruskal-Wallis test showed that there was no significant difference in microleakage at the enamel ( $P=0.834$ ) or dentin ( $P=0.894$ ) margins among the five immediate groups.

**Table 1:** Materials' brand names, composition and manufacturers' instructions

Material brand name	Composition	Manufacturer	Method of application
Scotchbond Etchant	37% Phosphoric acid	3M ESPE Dental Products, St. Paul, MN, USA	G1,3&5: 15 sec. etching+15 sec. rinsing G2&4: 15 sec. etching without rinsing
Adper Single Bond 2	Ethyl alcohol, Bis-GMA, HEMA, glycerol, 3-dimethacrylate, acrylic acid copolymer, itaconic acid, diurethane dimethacrylate, water, colloidal filler	3M ESPE Dental Products, St. Paul, MN, USA	G 1-5: Two layers applied & cured
Filtek Z-350	Bis-GMA, UDMA, TEGDMA, ethyl methacrylate, inorganic fillers	3M ESPE Dental Products, St. Paul, MN, USA	G 1-5: Two oblique increments applied in cavity and cured
Consepsis 2%	2% Chlorhexidine	Ultradent Products, South Jordan, UT, USA	G1&2: Applied 35 sec. before etching G3&4: Applied 35sec. after etching

**Table 2:** Microleakage scores of the enamel and dentin margins with and without thermocycling

Groups	score margin	Immediate N(%)				score margin	Thermocycled N(%)			
		0	1	2	3		0	1	2	3
G1	E	14(87.5)	2(12.5)	0	0	E	12(75.0)	4(25.0)	0	0
	D	3(18.8)	11(68.8)	2(12.5)	0	D	0	2(12.5)	12(75.0)	2(12.5)
G2	E	14(87.5)	2(12.5)	0	0	E	11(68.8)	5(31.3)	0	0
	D	5(31.3)	8(50.0)	3(18.8)	0	D	0	2(12.5)	11(68.8)	3(18.8)
G3	E	15(93.8)	1(6.3)	0	0	E	13(81.3)	3(18.8)	0	0
	D	5(31.3)	9(56.3)	2(12.5)	0	D	1(6.3)	8(50.0)	6(37.5)	1(6.3)
G4	E	13(81.3)	3(18.8)	0	0	E	13(81.3)	3(18.8)	0	0
	D	5(31.3)	9(56.3)	2(12.5)	0	D	3(18.8)	10(62.5)	3(18.8)	0
G5	E	13(81.3)	3(18.8)	0	0	E	11(68.8)	5(31.3)	0	0
	D	3(18.8)	10(62.5)	3(18.8)	0	D	0	3(18.8)	7(43.8)	6(37.5)

However, among the five thermocycled groups, a significant difference existed in microleakage at the dentin margin ( $P < 0.001$ ) while the difference in microleakage at the enamel margin was no significant ( $P = 0.859$ ). Pairwise comparisons by Dunn test (Table 3) revealed that in thermocycled groups, a significant difference existed between G1 (CHX+rinse+etching) and G4 (etching+CHX) ( $P = 0.002$ ), G2 (CHX+etching) and G4 ( $P = 0.001$ ) and G5 (control) and G4 ( $P < 0.001$ ). Mann-Whitney U test was used to compare microleakage in immediate and thermocycled groups. In G1 (CHX+rinse+etching) ( $P < 0.001$ ), G2 (CHX+etching) ( $P < 0.001$ ), G3 (etching+CHX+rinse) ( $P = 0.019$ ) and G5 (control) ( $P < 0.001$ ) greater microleakage was observed at the dentin margins of thermocycled specimens compared to non-thermocycled samples.

**DISCUSSION**

The present in-vitro study assessed the marginal sealing ability of CHX with different application protocols in class V composite restorations. Based on the results of the present study, various application protocols of 2% CHX did not show any adverse effect on immediate sealing ability of Adper Single Bond 2 total etch adhesive system.

Thus, the first hypothesis was supported. This result was supported by other studies [7,9]. The most probable explanation for this finding is that application of CHX before or after etching can improve the tooth surface energy and wettability of adhesive [18]. After 10,000 thermal cycles, the groups, which received CHX application showed greater microleakage compared to the immediate groups. Thus, the second hypothesis was not supported.

**Table 3:** Pairwise comparisons of the groups after thermocycling

Groups	G1 (CHX+rinse+etching)	G2 (CHX+etching)	G3 (etching+CHX+rinse)	G4 (etching+CHX)	G5 (control)
G1 (CHX+rinse+etching)	-	$P > 0.999$	$P = 0.294$	$P = 0.002^*$	$P > 0.999$
G2 (CHX+etching)	-	-	$P = 0.169$	$P = 0.001^*$	$P > 0.999$
G3 (etching+CHX+rinse)	-	-	-	$P > 0.999$	$P = 0.059$
G4 (etching+CHX)	-	-	-	-	$P < 0.001^*$
G5 (control)	-	-	-	-	-

\*Statistically significant ( $P < 0.05$ )

Although the reduction in microleakage in CHX groups was greater than that in the control group, the results showed that CHX was unable to perfectly prevent microleakage in long-term. According the results of this study, after thermocycling there were significant differences between G1 (CHX+rinse+etching) and G4 (etching+CHX), G2 (CHX+rinse+etching) and G4, and G5 (control) and G4. In this study, Consepis CHX solution was applied for its antibacterial activity to eliminate residual bacteria, as reported in other studies [12,19]. The least microleakage score was noted in G4 (etching+CHX) in our study. The other important issue associated with application of CHX is its inhibitory effect on MMPs, which are responsible for bond failure in long-term [20]. With regard to long term bond failure of E & R adhesives, two hypotheses have been suggested: First, application of low pH phosphoric acid for dentin etching can partially denature MMPs. Mild acids like those incorporated in E & R adhesives have the ability to activate new MMPs [21]. Furthermore, naked collagen fibrils, which are present at the bottom of the hybrid layer as a result of incomplete resin infiltration, are susceptible to MMP degradation [22]. Moreover, most simplified adhesives act as a semi-permeable membrane after curing. Breschi et al, [6] assumed that water can pervade into the adhesive interface and subsequently activate MMPs, leading to degradation of infiltrated collagen fibrils. It seems that collagenases need water to be activated for hydrolysis [23]. Due to water sorption mechanism, some of the hydrophilic monomers are removed from the hybrid layer; thus, the amount of exposed collagen fibrils will increase. This, in turn, will lead to enzymatic cleavage [24]. In case of E & R adhesives, decreased infiltration of resin may result in formation of unprotected collagen fibrils at the bottom of hybrid layer, which are also susceptible to hydrolytic degradation [21,22]. A scanning electron microscopic study reported

that deposition of CHX solution debris on the surface and within the etched dentinal tubules had no negative effect on the shear bond strength of composite to dentin when All-Bond 2 adhesive system was used [25]. This finding was in accordance with the results of the present study indicating that these deposits do not interfere with the bonding procedure of Adper Single Bond 2 and thus, do not affect the immediate microleakage scores. De Castro et al, [18] and Say et al, [26] showed that application of CHX before or after acid-etching had no negative effect on bond strength to dentin, which is consistent with the results of the present study. In addition, the effect of CHX on initial sealing ability of adhesive was in line with the results of Chang and Shin [7] who reported that CHX pretreatment did not affect the in-vitro bond strength of the tested specimens during the immediate testing period. However, after thermocycling (10,000 thermal cycles represent one year of clinical service), their results showed a significant increase in microleakage in groups that received CHX application before acid etching. On the contrary, groups that received CHX application after acid etching showed lower microleakage scores than the control group especially in no rinse groups. Breschi et al, [6] hypothesized that preservation of organic matrix may occur by the application of CHX at least for 30 seconds on denuded collagen after acid etching; whereas, CHX possibly continues to remain within collagen fibrils even though filling of interfibrillar spaces occurs with resin during the bonding process. They concluded that beneficial effects of CHX on protection of bond strength may last for more than 12 months [6]. On the other hand, increasing the tooth surface by acid etching and proper application of CHX with a strong positive ionic charge can increase the surface free energy of enamel and maybe dentin [7]. Thus, it seems that CHX application after acid etching of dentin could improve the wettability of dentin primers, leading to better

adhesion [18]. According to the results of this study, after thermocycling, microleakage score was high in G3 (etching+CHX+rinse). On the contrary, G4 (etching+CHX) showed the same microleakage score as that of groups before thermocycling. These results were in agreement with those of Carrilho et al, [15] who showed that CHX acts as a MMPs inhibitor when applied prior to bonding without rinsing. Thus, it preserves dentin bond strength [15]. In fact, the naked collagen fibrils are accessible to CHX in the mentioned protocol. Consequently, they are sealed with adhesive resins. This procedure could preserve them in a better way. Contrary to the current study results, some studies have reported interference of CHX with bonding procedure and consequent reduction in bond strength or increase in microleakage particularly in use of self-etching adhesive systems [17,27]. It seems that cavity disinfectants applied to dentin surfaces are resistant to acid conditioning. Moreover, this acid-resistant layer might inhibit the weak acidic primers to effectively demineralize dentin and prevent the hydrophilic resin to impregnate the dentinal surface [27]. Considering the antimicrobial activity of CHX [5], its chelating action and bond to several proteins [11] and no interference with the bonding process, it would be beneficial to preserve the collagen fibrils in hybrid layer by application of CHX without rinsing after acid etching of dentin in order to prevent degradation over time. In addition, since CHX has high water content, it can rehydrate the dried and demineralized dentin, and decrease the technical sensitivity of total etch adhesives. Nevertheless, more in vitro and in vivo studies are required to assess the effects of various concentrations of CHX on marginal seal and bond strength over time.

## CONCLUSION

Within the limitations of this study, the results showed that application of CHX after etching had

no adverse effect on microleakage scores in all groups. Thermocycling did not have any effect on microleakage of CHX groups.

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