

Effects of Three Hemostatic Agents on Microleakage of Cervical Composite Resin Restorations Bonded to Simulated Caries-Affected Tooth Substrate Using One-Step Self-Etch Adhesive

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Abstract

Background and Aim: This study aimed to evaluate the microleakage of cervical restorations with the use of a one-step self-etch adhesive after application of three different hemostatic agents.

Materials and Methods: In this in vitro study, 96 cervical cavities were prepared on 24 intact and 24 demineralized teeth. Forty-eight class V cavities with sound tooth substrate were assigned to groups 1 to 4, and 48 cavities with demineralized tooth substrate were assigned to groups 5 to 8. In groups 1 and 5, no hemostatic agent, in groups 2 and 6, Viscostat, in groups 3 and 7, Viscostat Clear, and in groups 4 and 8, trichloroacetic acid (TCA) were used. After composite resin filling and thermocycling, the teeth were immersed in dye, sliced, and dye penetration was scored under a microscope. Data were analyzed with the Kruskal-Wallis and Mann-Whitney tests.

Results: The mean enamel microleakage values were significantly different among the groups ($P=0.027$); however, there was no significant difference in the mean dentin microleakage values between the study groups ($P=0.072$). Significant differences were also noted in enamel marginal leakage between groups 1 and 3, 1 and 5, 3 and 8, and 5 and 8 ($P<0.05$). No significant difference was observed in microleakage of groups with normal dentin and groups with simulated caries-affected dentin ($P=0.063$).

Conclusion: Within the limitations of this study, Viscostat Clear exhibited the greatest enamel microleakage. There were no significant differences in dentin microleakage between the study groups; however, among the groups with simulated caries-affected dentin, TCA showed lower microleakage.

Key Words: Hemostatics, Dental Leakage, Dentin-Bonding Agents, Dental Cements

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Introduction

Despite the great advances in restorative dentistry, microleakage and subsequent discoloration of margins result in tooth hypersensitivity and recurrent caries, leading to eventual failure of composite restorations [1]. Microleakage is defined as the passage of fluids containing ions, molecules and microorganisms through the restoration-tooth interface, which is not clinically detectable. Many studies have shown that pulpal irritation is predominantly the result of microleakage which is the main cause of recurrent caries, pulpal inflammation, and pulp necrosis [2,3]. Various reasons have been reported for the microleakage of composite resin restorations, including polymerization shrinkage, difference between the coefficients of thermal expansion of composite resin and tooth structure, lack of self-sealing mechanism, and occlusal forces [4]. Additionally, lack of complete adhesion and bonding of composite resin to tooth structure is considered to be one of the most important etiologic factors for microleakage. The quality of adhesion between the composite resins and dentinal walls depends on proper interaction between the resin and collagen fibers [5].

Isolation is not always possible in cervical restorations, and sometimes trauma to the gingiva leads to bleeding during restorative procedures [4,7]. Contamination with blood and gingival crevicular fluid interferes with proper penetration of resin into the collagen fibers and compromises the bonding [5,6]. In cervical restorations with gingival margins apical to the cemento-enamel junction, complete isolation is not always possible. In these sites, hemorrhage usually occurs during restorative procedures due to gingival injuries, and in some cases crevicular fluid released because of gingival inflammation contaminates the surfaces of the prepared teeth [4,7].

One successful strategy to control bleeding is to use hemostatic agents before restorative procedures. [8]. These materials are divided into two categories based on their pharmacological action: astringents (blood clotting agents) and vasoconstrictors (adrenergic agents and vasoconstrictors).

Application of these agents raises a question whether contamination with hemostatic agents can affect bonding to tooth structure [9].

Aluminum chloride (25%), ferric sulfate (20%) and trichloroacetic acid (TCA) (35%) are some of the routinely used hemostatic agents in the field of restorative dentistry [9,10]. TCA, which precipitates in the form of proteins and is an acidic chemical caustic agent with a pH of 1 is mainly used for decalcification and fixation of tissues in microscopic studies, and is also used as a hemostatic agent in medicine [8,11].

Currently, the self-etch strategy, i.e. the use of an adhesive agent with no separate etching of the substrate, has become popular due to its ease of use [2]. Recently, all-in-one adhesive systems were introduced to the market. These systems have facilitated the bonding procedure; because there is no need for etching, rinsing and drying, and they significantly decrease the time needed for a direct composite restoration. This merit has resulted in increasing use of these adhesives [12,13]. In addition, these systems are not as technique-sensitive as total-etch systems and prevent the formation of a wide demineralized area [14]. However, considering the effect of the smear layer on the adhesion of self-etch adhesive systems [15,16], removal of the smear layer with hemostatic agents might negatively affect the bonding mechanism of such systems.

A study by Kaphasuk et al. showed that the use of hemostatic agents resulted in a decrease in bond strength of self-etch adhesives [17]. A study by Mohammadi et al. showed that contamination of cervical class V cavities with aluminum chloride during the application of one-step self-etch adhesives clearly increased the microleakage of composite resin restorations [18]. Kimmes et al reported that Viscostat Plus hemostatic agent (22% ferrous chloride) and Viscostat (20% ferrous sulfate) had no effect on shear bond strength in use of a total-etch adhesive system [5].

Today, extensive cavity preparation has been replaced by conservative preparation, and great attempts are made to preserve the maximum amount of tooth structure. In conservative cavity preparation, only the external layer of

carious dentin, which is infected and necrotic, is removed and the internal layer, which is called the affected dentin, remains. This dentin is demineralized but has the potential to become remineralized [19]. Consequently, when using composite resin restorations, the adhesive is bonded to the affected dentin. Besides, in preparation of cervical restorations, it is sometimes necessary to use hemostatic agents to control the hemorrhage since it may interfere with the bonding of self-etch adhesive to the adjacent dentinal layer. Moreover, some of these agents might cause some discolorations in restorations due to their dark color.

Considering the conservative approach to preserve the affected dentinal layer and the use of self-etch adhesive systems, the aim of this study was to evaluate the microleakage of cervical restorations with the use of one-step self-etch adhesive on simulated caries-affected dentin after applying Viscostat, Viscostat Clear and TCA as hemostatic agents.

Materials and Methods

In this in vitro study, 48 sound human third molar teeth with no cracks, abrasion or structural defects were selected (ethical code: #191165). The samples were cleaned with a brush and pumice paste, rinsed, and stored in 0.3% thymol solution before use. Then, the teeth were mounted in self-cure acrylic resin (Unifast III, GC Corp., Tokyo, Japan) and stored in cold water until the curing process was completed in order to control the thermal effects of the curing acrylic resin.

A total of 96 class V non-beveled cavities were prepared in the buccal and lingual surfaces of each sample using a diamond bur (Hi-Di, Dentsply Sirona, London, UK) mounted on a high-speed handpiece with the cervical margin 1 mm below the cemento-enamel junction. The occlusogingival and mesiodistal dimensions of the cavities were 2 mm, with a depth of 1.5 mm. All the cavity walls were butt-joint with no bevels. Afterwards, 48 of these cervical cavities underwent mineralization/demineralization cycles to induce dental caries. Each mineralization/demineralization cycle consisted of 3 h of immersion in a

demineralizing solution (156.25 mL/tooth), followed by 45 h of immersion in a remineralizing solution (78.125 mL/tooth). The samples underwent eight remineralization/demineralization cycles, each lasting for 48 h. The demineralizing and the remineralizing solutions were replaced by new solutions after the 4th cycle and before each new cycle, respectively [20]. Table 1 presents the chemical composition of the demineralizing and remineralizing solutions.

The 48 cavities with sound dentin (N group) were assigned to groups 1 to 4, and 48 cavities with simulated caries-affected dentin (CAD group) were assigned to groups 5 to 8. Therefore, 8 groups (n=12) were evaluated in the present study (Table 2).

In groups 1 and 5, which served as controls, no hemostatic agents were used. In groups 2 and 6, Viscostat (Ultradent Products, South Jordan, USA) was applied for 2 min according to the manufacturer's instructions. Then, each tooth was rinsed with water spray for 30 s and dried with oil-free air. In groups 3 and 7, Viscostat Clear (Ultradent Products, South Jordan, UT, USA) and in groups 4 and 8, TCA were used in the same way as groups N/Vis and CAD/Vis. The chemical composition of Viscostat is 20% ferric sulfate and that of Viscostat Clear is 25% aluminum chloride [9].

Subsequently, a mild one-step self-etch adhesive (Clearfil SE Bond, Kuraray, Okayama, Japan) was applied according to the manufacturer's instructions (application of the adhesive for 20 s, drying with mild air spray and light-curing for 10 s). This adhesive is an ultra-mild adhesive with a pH of 2.7 [21]. Then, all cavities were restored with A3 shade of a light-cure composite resin (APX, Kuraray, Tokyo, Japan), which was cured for 40 s using a light-curing unit (Coltolux 50, Mod.C7950, Coltene/Whaledent Inc., Cuyahoga Falls, OH, USA). The restorations were finished and polished (3M ESPE, St. Paul, MN, USA) and stored at 37°C for 24 h. After 1000 thermal cycles, the teeth were stored in 2% fuchsin solution for 24 h and were then sectioned in half parallel to the longitudinal axis of the tooth using a 0.3 mm-thick diamond-coated cutting

Table 1. Chemical composition of solutions used in the study to induce dental caries

Demineralizing solution (pH = 4.5)	2.2 mM calcium (CaCl ₂) 2.2 mM phosphate (NaH ₂ PO ₄) 0.05 M sodium acetate 0.05 M acetic acid 1 ppm fluoride (NaF)
Remineralizing solution (pH = 7.0)	1.5 mM calcium (CaCl ₂) 0.9 mM phosphate (NaH ₂ PO ₄) 0.15M KCl 0.1M Tris buffer 10 ppm fluoride (NaF)

Table 2. Study groups in terms of dentin type and hemostatic agent used

(N: normal dentin, N/Vis: Normal dentin, Viscostat, N/Vis. Clear: Normal dentin, Viscostat Clear, N/TCA: Normal dentin, Trichloroacetic acid, CAD/-: Caries affected dentin, CAD/Vis: Caries affected dentin, Viscostat, CAD/Vis.Clear: Caries affected dentin, Viscostat Clear, CAD/TCA: Caries affected dentin, Trichloroacetic acid.)

Dentin Type	Group Number	Group Name/definition	Hemostatic Agent
Normal dentin	1	N/-	-
	2	N/Vis	Viscostat
	3	N/Vis.Clear	Viscostat Clear
	4	N/TCA	TCA
	5	CAD/-	-
Simulated caries-affected- dentin	6	CAD/Vis	Viscostat
	7	CAD/Vis.Clear	Viscostat Clear
	8	CAD/TCA	TCA

disc (Buehler Isomet Diamond Wafering Blade, Dusseldorf, Germany). Penetration of dye was evaluated and scored under a stereomicroscope (SMP-200, HP, Palo Alto, CA, USA) at ×32 magnification.

The following classification was used to score the penetration of dye:

Score 0: No penetration of dye

Score 1: Penetration of dye up to 1/3 of the cavity depth

Score 2: Penetration of dye up to 2/3 of the cavity depth

Score 3: Penetration of dye for more than 2/3 of the cavity depth

Data were analyzed with the Kruskal-Wallis and Mann-Whitney tests using SPSS 22 (SPSS Inc., Chicago, IL, USA) at a significance level of 0.05.

Results

Tables 3 and 4 present the frequency of enamel and dentin microleakage in the study groups and the mean and mean rank of enamel and dentin microleakage, respectively.

According to the Kruskal-Wallis test, the mean

Table 3. Frequency of enamel and dentin microleakage scores in the study groups

(N: normal dentin, N/Vis: Normal dentin, Viscostat, N/Vis. Clear: Normal dentin, Viscostat Clear, N/TCA: Normal dentin, Trichloracetic acid, CAD/-: Caries affected dentin, CAD/Vis: Caries affected dentin, Viscostat, CAD/Vis.Clear: Caries affected dentin, Viscostat Clear, CAD/TCA: Caries affected dentin, Trichloracetic acid.)

Groups (n=12)	Group name or definition	Enamel margin microleakage				Dentin margin microleakage				Total
		0	1	2	3	0	1	2	3	
1	N/-	10 83.3%	0 0.0%	1 8.3%	1 8.3%	0 0.0%	0 0.0%	0 0.0%	12 100.0%	12
2	N/Vis	8 66.7%	1 8.3%	0 0.0%	3 25.0%	1 8.3%	2 16.7%	2 16.7%	7 58.3%	12
3	N/Vis.Clear	3 25.0%	3 25.0%	3 25.0%	3 25.0%	0 0.0%	1 8.3%	2 16.7%	9 75.0%	12
4	N/TCA	5 41.7%	6 50.0%	1 8.3%	0 0.0%	2 16.7%	1 8.3%	1 8.3%	8 66.7%	12
5	CAD/-	3 25.0%	4 33.3%	0 0.0%	5 41.7%	0 0.0%	0 0.0%	0 0.0%	12 100.0%	12
6	CAD/Vis	6 50.0%	4 33.3%	1 8.3%	1 8.3%	1 8.3%	0 0.0%	1 8.3%	10 83.3%	12
7	CAD/Vis.Clear	7 58.3%	2 16.7%	0 0.0%	3 25.0%	0 0.0%	1 8.3%	1 8.3%	10 83.3%	12
8	CAD/TCA	10 83.3%	1 8.3%	0 0.0%	1 8.3%	0 0.0%	1 8.3%	0 0.0%	11 91.7%	12

Table 4. Mean and mean rank of enamel and dentin microleakage in the study groups

(N: normal dentin, N/Vis: Normal dentin, Viscostat, N/Vis. Clear: Normal dentin, Viscostat Clear, N/TCA: Normal dentin, Trichloracetic acid, CAD/-: Caries affected dentin, CAD/Vis: Caries affected dentin, Viscostat, CAD/Vis.Clear: Caries affected dentin, Viscostat Clear, CAD/TCA: Caries affected dentin, Trichloracetic acid.)

Group Name or definition	Group number	Enamel microleakage		Dentin microleakage	
		Mean	Mean rank	Mean	Mean rank
N/-	1	.4167	43.29	3.0000	144.50
N/Vis	2	.8333	60.96	2.2500	113.33
N/Vis.Clear	3	1.5000	83.25	2.6667	129.21
N/TCA	4	.6667	54.38	2.2500	114.42
CAD/-	5	1.5833	90.67	3.0000	144.50
CAD/Vis	6	.7500	57.13	2.6667	130.29
CAD/Vis.Clear	7	.9167	64.42	2.7500	133.75
CAD/TCA	8	.3333	41.63	2.8333	138.29

enamel microleakage values were significantly different between the study groups ($P=0.027$). However, there were no significant differences in the mean dentin microleakage values between the study groups ($P=0.072$).

Pairwise comparisons of enamel microleakage between the eight study groups with the Mann-Whitney test showed significant differences between group 1 (control, sound dentin) and group 3 (sound dentin, Viscostat Clear), group 1 and group 5 (control, mineralized dentin), group 3 and group 8 (demineralized dentin, TCA), and groups 5 and 8, with P-values of 0.024, 0.017, 0.014 and 0.012, respectively.

Based on the results of the Kruskal-Wallis test, there were no significant differences in microleakage between the control groups and the three hemostatic agents used in the present study ($P>0.05$).

The Mann-Whitney test showed no significant difference in microleakage of the groups with normal dentin and groups with simulated caries-affected dentin ($P=0.063$).

Discussion

Prevention of contamination of prepared cavity surfaces before the application of composite resin and adhesive system is absolutely crucial to achieve a successful and durable bond. However, in many cases, it is difficult to use a rubber dam and therefore contamination with blood or saliva is inevitable [22]. The most common technique to control hemorrhage and decrease the gingival crevicular fluid flow is to use local hemostatic agents [4]. The question is whether contamination with hemostatic agents can affect bonding to tooth structure or not [9]. Self-etch adhesive systems can be applied in a short time due to the limited number of components and procedural steps; therefore, they decrease the risk of blood contamination [6,10]. In addition, they prevent the accidental contact of the cavity margins with phosphoric acid during irrigation [10]. Therefore, in this study, a one-step self-etch adhesive was used.

In the present study, the mean microleakage of dentin was higher than that of enamel. An increase in microleakage at the gingival margin

in cavities apical to the cemento-enamel junction might be attributed to polymerization shrinkage of composite resin towards the enamel margin with a stronger bond [4]. Kumar et al. evaluated the microleakage of two bonding agents from two different generations in presence of different surface contaminations [23]. They used Single Bond (3M) and I Bond (HeraeusKuraray) and evaluated microleakage in presence of different contaminations. In contrast to the results of the present study, in their study, I Bond exhibited more microleakage at the enamel margins compared with the gingival margins, which might be attributed to the chemical composition of I Bond, which is acetone-based with a pH of 2.4; it results in shallow etching and produces less resin tag complexes [4]. However, Clearfil SE Bond (Kuraray, Osaka, Japan) is an ultra-mild ethanol-based adhesive with a pH value of 2.7 that contains MDP monomer [21]. In the present study, enamel microleakage with the use of Viscostat Clear (group 3) was significantly higher than that in the control group (group 1).

Mohammadi et al. evaluated the effect of aluminum chloride (Hemostop, Dentsply) on the microleakage of gingival margins with the use of an all-in-one adhesive resin and reported a significant increase in microleakage at the gingival margin [18]. A decrease in sealing ability and an increase in microleakage might be attributed to the removal of the smear layer, which affects the bonding mechanism of self-etch systems [17,18]. Previous studies have shown that hemostatic agents are very acidic, with a pH value of 0.7-3. The acidic aluminum chloride solution at a concentration of 20-25% demineralizes the dentin surface to different degrees with different patterns. It has been shown that some demineralization is evident on the dentin surface exposed to 21.3% aluminum chloride for 5 min, with complete removal of the smear layer [22,3]. In addition, surface contamination with aluminum chloride decreases the demineralization after the use of a self-etch system, which might be attributed to the replacement of calcium in hydroxyapatite with the hemostatic agent and formation of an

insoluble complex. Therefore, demineralization is limited with the use of weak self-etch systems [17,18]. In contrast, Kuphasuk et al. [17] evaluated the effect of aluminum chloride hemostatic agent on total-etch systems and reported no significant difference between the bond strength of normal and contaminated dentin. This difference might be due to the high acidity of phosphoric acid and its higher demineralization capacity [17,18]. In the study by Kumar et al, when the cavities were exposed to a hemostatic agent (Viscostat), I Bond exhibited more microleakage at both margins compared with the control group, and more microleakage was observed at the enamel margins compared with the use of Single Bond [23]. An increase in microleakage might be due to the fact that Viscostat is a viscous gel containing ferric sulfate. Its viscous form makes it difficult to remove it from the surface. The coagulated proteins and the remnants of ferric sulfate might prevent the penetration of the bonding agent into the etched enamel and dentinal tubules [4]. Some studies have suggested rinsing with water (Consepsis) or air abrasion with aluminum oxide particles to clean the surfaces contaminated with hemostatic agents [4,7].

In the present study, there was a significant difference between groups 1 and 5, and enamel microleakage in the control group with simulated caries-affected dentin was higher than that in the control group with normal dentin. This might be due to the accidental and inevitable contact of enamel margins with the demineralizing agent, which leads to the loss of inorganic ions from the enamel surface, interfering with poor self-etch bonding systems. Further studies are necessary on this subject.

In the present study, the minimum amount of microleakage at the enamel margin was recorded with the use of TCA in the group with simulated caries-affected dentin (group 8), with a significant difference with the control group with simulated caries-affected dentin (group 5). There was no significant difference between group 8 and the control group with normal dentin (group 1). A study by Khoroushi and Tavasoli showed that TCA, as a hemostatic

agent, not only did not decrease the bond strength of composite resin to enamel, but increased it when contacted the tooth surface. They attributed this to the acidity of TCA and also to the fact that it does not leave any deposits after being rinsed with water; therefore, it does not interfere with bonding [8]. In addition, this finding is consistent with the results reported by Lewinstein and Rotstein on the effects of etching and conditioning with TCA [11].

In the present study, despite the fact that no significant differences were observed in dentin microleakage, there was greater microleakage in the control groups, which might be attributed to the presence of a thick smear layer because coarse burs had been used to prepare the cavities. Self-etch adhesives dissolve the smear layer to some extent [22,24], and some adhesives have a chemical reaction with the smear layer through their monomers such as MDP. Weaker adhesives result in greater interference of the smear layer with bonding. Use of extra-fine diamond burs has been recommended to finish the cavities with the use of mild and ultra-mild self-etch adhesives [25]. Hemostatic agents are very acidic and have the capacity to remove a part of the smear layer and demineralize dentin surfaces to some extent [8]. Finally, since the present study was an in vitro study and it was not possible to carry out evaluations in presence of contamination with blood or gingival crevicular fluid, further comprehensive clinical studies are necessary. In addition, it is advisable to carry out studies with larger sample size.

Conclusion

Clinically, to achieve a successful bonding, contamination should be prevented through isolation, and in case of contamination and use of hemostatic agents, the surfaces should be thoroughly cleaned. Under the conditions of the present study, Viscostat Clear exhibited the greatest microleakage and TCA resulted in lower enamel microleakage in the group with simulated caries-affected dentin. There was no significant difference in dentin microleakage between the study groups. Further studies are

recommended with larger sample size.

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