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Original Article

Combination Effect of Hemostatic and Disinfecting Agents on Micro-leakage of Restorations Bonded with Different Bonding Systems

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Abstract

Statement of Problem: Hemostatic agents may affect the micro-leakage of different adhesive systems. Also, chlorhexidine has shown positive effects on micro-leakage. However, their interaction effect has not been reported yet.

Objectives: To evaluate the effect of contamination with a hemostatic agent on micro-leakage of total- and self-etching adhesive systems and the effect of chlorhexidine application after the removal of the hemostatic agent.

Materials and Methods: Standardized Class V cavity was prepared on each of the sixty caries free premolars at the cemento-enamel junction, with the occlusal margin located in enamel and the gingival margin in dentin. Then, the specimens were randomly divided into 6 groups (n = 10) according to hemostatic agent (H) contamination, chlorhexidine (CHX) application, and the type of adhesive systems (Adper Single Bond and Clearfil SE Bond) used. After filling the cavities with resin composite, the root apices were sealed with utility wax. Furthermore, all the surfaces, except for the restorations and 1mm from the margins, were covered with two layers of nail varnish. The teeth were immersed in a 0.5% basic fuschin dye for 24 hours, rinsed, blot-dried and sectioned longitudinally through the center of the restorations bucco- lingualy. The sections were examined using a stereomicroscope and the extension of dye penetration was analyzed according to a non-parametric scale from 0 to 3. Statistical analysis was performed using Kruskal-Wallis test and Mann-Whitney U-test.

Results: While ASB group showed no micro-leakage in enamel, none of the groups showed complete elimination of micro-leakage from the dentin. Regarding micro-leakage at enamel, and dentin margins, there was no significant difference between groups 1 and 2, 1 and 3, and 2 and 3 ($p > 0.05$). A significantly lower micro-leakage at the enamel and dentin margins was observed in group 3, compared to group 6. No significant difference was observed between groups 4 and 5 in enamel ($p = 0.35$) and dentin ($p = 0.34$). Group 6 showed significantly higher micro-leakage, compared to group 4 and 5 ($p < 0.05$).

Conclusions: Hemostatic agent contamination had no significant effect on micro-leakage of total- and self-etching adhesive systems. Application of chlorhexidine after the removal of hemostatic agent increased micro-leakage in self-etching adhesives but did not affect when total-etching was used.

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Introduction

Micro-leakage is the transition of fluids, bacteria, ions or molecules between a cavity wall and the restorative material [1]. In composite restorations, micro-leakage can be caused by polymerization shrinkage, the differences in the coefficient of thermal expansion between tooth structure and the material, and occlusal loads [2]. Contamination in different stages of bonding procedure may also cause micro-leakage [3-5]. Clinically, it can lead to staining around the margins of restorations, margin deterioration, poor aesthetics, post-operative sensitivity, secondary caries, restoration failure, pulpal pathology, and unfavorable effects on clinical performance of composite restorations [6-8]. In most of the current literature on adhesive techniques and materials, the focus is on the elimination of leakage because of its importance for the long-term success of restorations [1].

In order to achieve a durable bond, moisture control is essential. However, isolation against saliva and blood contamination is sometimes difficult, especially at and below the gingival margin [9]. Saliva and blood contamination can reduce the dentin bond strength of total- and self-etching adhesive systems [10-12]. Clinicians may rely on hemostatic agents to avoid blood contamination, particularly near or at the gingival margin where blood contamination is more probable [13]. Hemostatic agents are substances that can be used on hemorrhagic gingival tissues before placing restorations [14-15]. Aluminium chloride with a concentration between 5 and 25% is one of the most frequently used hemostatic agents which, with its minimal systemic effects, can precipitate proteins, constrict blood vessels and extract fluid from tissues [14]. Hemostatic agents are highly acidic and are able to remove smear layer and cause some degree of demineralization [15].

Although the effect of hemostatic agents on micro-leakage and bond strength has been evaluated in several studies, they have reached different results [13,15,16]. If viable microorganisms remain after cavity preparation, micro-leakage may cause more problems which can result in secondary caries [6]. According to some bacteriologic and histologic studies, only a small portion of teeth is sterile after cavity preparation [7]. Proliferation of residual bacteria from the smear layer occurs even when there is a good seal from the oral cavity [17]. This problem can be magnified by micro-leakage of composite restoration at margins not ending on enamel [18]. Treating the cavity preparation with chlorhexidine may solve this problem to some extent. Chlorhexidine has a broad spectrum of action, mostly against Gram-positive bacteria, particularly Streptococcus mutans, which seems to be more sensitive [19]. Currently, chlorhexidine is used not only as an anti-microbial agent, but also as a potential adjuvant to establish a better bond to dentin [20]. However, owing to the lack of consensus existing over the effect of chlorhexidine [20,21], further studies are required in this regard .

Generally, bond degradation occurs because of resin elution and collagen fibrils alteration. Exposed collagen fibrils that are exposed in the hybrid bond layer, and are not completely infiltrated by resin are susceptible to degradation by matrix metalloproteinase (MMP) enzymes of the saliva and dentin [20,22].

MMPs that are host cell-derived proteolytic enzymes have a major role in tissue-destructive inflammatory diseases and are capable of degrading most of the extracellular matrix components, including different types of collagen [23,24]. Chlorhexidine can be used to prevent or minimize the auto-degradation of collagen matrices in incompletely resin-infiltrated dentin [25,26].

Considering the contrasting effects of hemostatic agents on micro-leakage, and the positive effects of chlorhexidine mentioned above, this study aimed to evaluate the effect of hemostatic agent contamination on micro-leakage of total- and self-etching adhesive systems and the effect of chlorhexidine application after the removal of the hemostatic agent.

The null hypothesis was that hemostatic agent contamination would not affect the micro-leakage of total- and self-etching adhesive systems and the application of chlorhexidine after the removal of hemostatic agent would not affect micro-leakage.

Materials and Methods

Sixty extracted non-carious human premolar teeth collected and stored at 4°C were used within one month of extraction. Standardized Class V

cavities (2 mm depth, 2 mm mesiodistal, 3 mm occlusogingival) were prepared at the cemento-enamel junction, with the occlusal margin located in enamel and the gingival margin in dentin. In the occlusal cavo surface margin, a bevel was made. Cavities were prepared using a #556 diamond fissure bur in a high-speed handpiece with water coolant. Then, they were randomly divided into 6 groups (n = 10) according to the following factors: Hemostatic agent contamination, chlorhexidine application, and the adhesive systems used. Group 1- Adper Single Bond system (ASB) (3M,ESPE,USA) was applied as recommended by the manufacturer (Table 1). Group 2- One drop of hemostatic agent solution (Hemostop,Dentsply. Argentina) was applied on the dentin surface for 2 minutes and rinsed for 30s and air-dried .Then, the cavity surface was etched with 37% phosphoric acid gel for 15s. After rinsing and air-drying, ASB was applied (H + ASB). Group 3- One drop of the hemostatic agent solution was applied into the cavity for 2 minutes, rinsed for 30s and air-dried. After that, CHX 0.2% solution was applied with a microbrush.The next steps were similar to group 1 (H+CHX+ASB). Group 4- Clearfil SE Bond system (CSB) (Kuraray,Japan) was applied as recommended by the manufacturer (Table 1). Group 5- One drop of hemostatic agent solution was applied into the cavity for 2 minutes and rinsed for 30s and air-dried. Then, CSB was applied (H+CSB).Group 6- One drop of hemostatic agent solution was applied into the cavity for 2 minutes and rinsed for 30s and air-dried. After that, CHX 0.2% solution was applied with a microbrush. Then, CSB was applied (H+CHX+CSB).

After the adhesive application and light curing for 20s with an LED light curing system (Demetron,Kerr)(1200 mW/cm2), the cavities were bulk filled with one increment of resin composite (Filtek Z250, 3M, ESPE, St Paul, MN, USA). Excess composite was removed with an explorer and then light cured with an LED for 20s. All procedures were performed by one operator at room temperature and during procedures, the cavities were kept moist. After the restoration, the samples were stored for 24 hours in distilled water.

Afterwards, the root apices were sealed with utility wax, and all the surfaces, except for the restorations and 1mm from the margins, were covered with two layers of nail varnish. The teeth were immersed in a 0.5% basic fuschin dye for 24 hours. Then, they were rinsed, blot-dried and sectioned longitudinally through the center of the restorations from the buccal to lingual surface with a water-cooled diamond saw (Leitz 1600, Wetzlar, Germany).

The sections were examined for dye penetration by two independent researchers using a stereomicroscope (Carl ZiessInc, Oberkochen, Germany) at 20× magnification. The extent of the dye penetration was analyzed according to a non-parametric scale from 0 to 3 (0 = no dye penetration, 1 = dye penetration less than 1/2 of the cavity depth, 2 = dye penetration more than 1/2 of the cavity depth, 3 = dye penetration spreading along the axial wall).

Statistical analysis was performed using Kruskal-Wallis H test and Mann-Whitney U-test. Significance level for all statistical tests was predetermined at $p < 0.05$.

Table 1: Adhesive Systems, Composition, and Application Mode

Adhesive Systems	Composition	Application mode
Adper Single Bond (3M, ESPE, St Paul, MN, USA)	Scotchbond etchant:37% phosphoric acid Adhesive:Bis-GMA, HEMA, dimethacrylates, polyalkenoic acid copolymer, initiators, water, and ethanol	1.Apply acid etch for 15s 2.Rinse for 15s. 3.Blot dry for 30s. 4.Apply one coat of adhesive for 10s. 5.Airdry for 10s at 20cm. 6.Light cure.
Clearfil SE Bond (Kuraray, Okayama, Japan)	Primer:MDP,HEMA,dimethacrylate Monomer,water,catalyst Bond:MDP,HEMA, dimethacrylate Monomer,microfiller,catalyst	1.Apply primer and leave for 20s. 2.Drythorouly with mild air flow. 3.Apply bond. 4.Gentle air flow. 5.Light cure.

Table 2: Micro-leakage scores of enamel and dentin

Group	Material	Enamelscores				Mean	Mediam	Dentinscores				Mean	Mediam
		0	1	2	3			0	1	2	3		
1	ASB	12	0	0	0	0	0	5	6	1	0	0.6	1
2	H+ASB	10	2	0	0	0.16	0	3	7	2	0	0.91	1
3	H+CHX+ASB	10	1	1	0	0.25	0	8	4	0	0	0.33	0
4	CSB	8	4	0	0	0.33	0	7	5	0	0	0.41	0
5	H+CSB	6	5	1	0	0.58	0.5	5	6	1	0	0.66	1
6	H+CHX+CSB	0	9	1	2	1.41	1	0	8	3	1	1.41	1

ASB=Adper Single Bond, CSB=Clearfil SE Bond, CHX= Chlorhexidine, H= Hemostatic

Results

Dye penetration scores for the enamel and dentinal margins are presented in Table 2. Whereas ASB group showed no micro-leakage in enamel, none of the groups showed complete elimination of micro-leakage in dentin. Pairwise multiple comparisons of the six groups were performed using the Mann-Whitney test between treatment groups for both enamel and dentinal margins.

There was no significant difference between groups 1 and 2 neither in enamel ($p = 0.51$) nor in dentin ($p = 0.41$). Moreover, there was no significant difference between groups 1 and 3 concerning enamel ($p = 0.51$), and dentin ($p = 0.26$). Also, no significant difference was seen between groups 2 and 3 regarding enamel ($p = 0.97$) and dentin ($p = 0.52$) margins. However, significantly lower micro-leakage at enamel and dentin margins was observed in group 3, compared to group 6 ($p < 0.05$).

No significant difference was observed between groups 4 and 5 in enamel ($p = 0.35$) and dentin ($p = 0.34$) margins. Group 6 showed significantly higher micro-leakage, compared to group 4. ($p < 0.05$) and group 5 ($p < 0.05$).

Discussion

In the first part of this study, we evaluated the effect of hemostatic agent contamination on the micro-leakage of total- and self-etching adhesive systems to dentin. As the findings of the study failed to reject the first part of the null hypothesis, it is concluded that hemostatic agent contamination had no significant effect on the micro-leakage of total- and self-etching adhesive systems. These findings are in line with a previous study performed by Arslan *et al*. that reported no significant changes in micro-leakage scores after the application of the hemostatic agent, with self-etching adhesives [13]. In another study, the researchers used Viscostat as a hemostatic agent and contrary to our findings; they reported significant increase in micro-leakage [3]. The difference in the results of that study and the present study may be attributed to differences in the hemostatic agents used.

The increased micro-leakage might be because of the fact that Viscostat, contrary to Hemostop, is a viscous gel composed of ferric sulphate and the viscosity makes its removal from the dentin surface harder, and may lead to coagulation of proteins present in the dentinal fluid. The coagulated proteins and residues of ferric sulfate may result in the inhibition of the infiltration of the bonding agent into the etched enamel surfaces and dentinal tubules.

Evaluation of the effect of chlorhexidine application after the removal of hemostatic agent revealed that the use of chlorhexidine after astringent removal did not have any significant effects on micro-leakage when ASB was used as adhesive system and the second part of the null hypothesis was accepted for ASB.

But regarding CSB, chlorhexidine application increased micro-leakage and the null hypothesis was rejected in this regard.

For deciding the step in which chlorhexidine is applied, in some studies, chlorhexidine has been used after etching [26,27]. The rationale for the application of chlorhexidine in this step is the

ability of chlorhexidine to inhibit enzyme activities directly; inhibiting the inherent collagenolytic activity of mineralized dentin would reinforce the bond of resin composite to dentin. In some other investigations, chlorhexidine has been used before etching with the purpose of the disinfection and cleansing of the dentin surface [21,28]. Also, according to some other investigators, there is no significant difference between the application of chlorhexidine before or after etching of dentin [29,30]. In this study, chlorhexidine was used after removing the hemostatic agent and before etching in order to disinfect the dentin surface and remove the microorganisms and thus to reduce the probability of recurrent caries and restoration failure as well as eliminating the remnants of hemostatic agent that could interfere with the bond of composite to dentin.

In a study by Sung *et al.*, the researchers concluded that application of chlorhexidine had no significant effect on micro-leakage of total-etching system [31]. Siso *et al*. reported that chlorhexidine solution had no effect on micro-leakage of composite restorations [32].

Although a number of studies obtained different results after chlorhexidine use [31-33], some other studies reported findings similar to ours. For instance, Tulunoglu *et al*. concluded that chlorhexidine solution had an adverse effect on self-etching adhesive systems and produced higher micro-leakage [33]. Singla *et al.* also reported higher micro-leakage when using chlorhexidine with self-etching adhesives [34].

Further in vitro and in vivo studies are recommended to improve the understanding of the interaction effect of different hemostatic agents and chlorhexidine with bonding systems which have different acidities.

Conclusions

Considering the limitations of this in vitro study, we found that hemostatic agent contamination had no significant effect on micro-leakage of total- and self-etching adhesive systems. Application of chlorhexidine after the removal of hemostatic agent could increase micro-leakage in self-etching adhesives but did not affect the micro-leakage when using total-etching adhesive systems.

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Conflict of Interest: None declared.

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