



## Effect of Different Cleansing Protocols on Bond Strength of Composite Resin to Dentin Contaminated with Hemostatic Agent: An In Vitro Study

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### ABSTRACT

**Objectives:** Inadequate removal of the hemostatic agent can adversely affect the bond strength of restorations to the tooth structure. This study aimed to assess the effect of different cleansing protocols on the shear bond strength (SBS) of an etch-and-rinse adhesive to dentin contaminated with aluminum chloride hemostatic agent.

**Materials and Methods:** In this experimental study, the mid-coronal dentin of 96 premolars was exposed. They were contaminated with a hemostatic agent (ViscoStat Clear) and then randomly divided into 7 groups (n=12). One group served as the control. The groups underwent various cleaning methods as follows: water spray, aluminum oxide particles (27 $\mu$  diameter), a slurry of pumice with water, GC dentin conditioner (GCDC), sodium hypochlorite 2% (SHC), and chlorhexidine 2% (CHX). Composite cylinders were then fabricated and bonded to the surfaces using Scotchbond Multi-Purpose etch-and-rinse bonding agent. After thermocycling (10,000 cycles), the SBS was measured using a universal testing machine. Data were analyzed using one-way analysis of variance (ANOVA) and Tukey HSD (honestly significant difference) test, and the significance level was set at 0.05.

**Results:** The SBS of the groups was significantly different (P=0.036). The SBS was the highest in the CHX and SHC groups, and the lowest SBS was related to the control group and GCDC groups. The difference between other groups was not significant (P=0.996).

**Conclusion:** CHX and SHC yielded the highest bond strength among the tested modalities for cleansing the ViscoStat Clear from the tooth surface.

**Keywords:** Adhesives; Aluminum Chloride; Dental Bonding; Hemostatics

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## INTRODUCTION

Hemostatic agents are commonly used in dental treatments to prevent gingival bleeding and provide efficient isolation. Aluminum chloride is a commonly used hemostatic agent in dentistry [1]. It is available in 5% to 25%

concentrations. It causes vasoconstriction and has insignificant systemic side effects. Among different hemostatic agents, aluminum chloride has the least side effects [2]. Certain products are available in the market for this purpose such as ViscoStat Clear, which is a

non-drip 25% aluminum chloride gel. It arrests minor bleeding and gingival crevicular fluid (GCF) rapidly and efficiently with no tissue damage or staining [3]. Hemostatic agents are hydrophilic and can contaminate the tooth structure and adhesives and negatively affect their bond strength. Reduction in bond strength of bonding agents to tooth structure following the use of hemostatic agents is a common concern for dentists since it can lead to caries recurrence and treatment failure [4,5].

Failure in the efficient removal of hemostatic agents from the tooth surface increases the microleakage and leads to caries recurrence and related complications [5]. Thus, several physical and chemical cleaning protocols have been proposed for removal of the hemostatic agent from the tooth surface, especially in cervical class V restorations [2-6].

Chlorhexidine (CHX) has been used for the removal of hemostatic agents and has shown successful results in increasing bond strength [2].

Physical cleansing methods commonly used for removal of hemostatic agents from the tooth structure include abrasion with pumice paste, use of hand instruments such as excavator, and sandblasting with different sizes of aluminum oxide particles [6]. The cleansing efficacy of some of these cleansing methods for the bond strength of some cements has been previously investigated [4-6]. However, the available literature on this topic is inconclusive [7,8]. Therefore, this study aimed to assess and compare the effect of various mechanical and chemical cleansing protocols on the bond strength of an etch-and-rinse adhesive to dentin contaminated with aluminum chloride hemostatic agent.

## MATERIALS AND METHODS

This in-vitro experimental study evaluated 96 premolars extracted for orthodontic purposes. The inclusion criteria were premolar teeth with no cracks, fractures, carious lesions, or previous restorations extracted within the past one month, which were selected using convenience sampling. They were cleaned from debris and tissue remnants and stored in a 0.5% phenol amine solution (Fisher

Chemical, Fair Lawn, NJ, USA) for 24 hours for disinfection [9,10] and were then stored in distilled water at 37°C until the experiment. The mesial and distal enamel was removed using a high-speed diamond saw (Leitz 1600; Wetzlar, Germany) under water irrigation to expose the mid-coronal dentin. After mounting the teeth in an auto-polymerizing acrylic resin block [1mm apical to the cemento-enamel junction (CEJ)], the exposed dentin surfaces were polished using a 600-grit silicon carbide paper under a water coolant for 30 seconds to achieve a standard smear layer. The teeth were then randomly divided into 8 groups (n=12) based on the cleansing protocol as follows:

In the control group, one drop of 25% aluminum chloride (ViscoStat Clear, Ultradent, UT, USA) was applied to the surface for 120 seconds. No cleaning was performed.

In group A, following the application of aluminum chloride as explained in the control group, tooth surfaces were rinsed with water for 60 seconds and dried by blotting with a cotton pellet.

In group B, following the application of aluminum chloride as explained in the control group, tooth surfaces were sandblasted using aluminum oxide particles with a 27 $\mu$  diameter under 40 Psi pressure from a 2mm distance for 10 seconds using a sandblaster (Vafaei, Tehran, Iran) and then rinsed with water for 60 seconds and dried by blotting with a cotton pellet.

In group C, following the application of aluminum chloride as explained in the control group, tooth surfaces were cleaned with a slurry of non-fluoridated pumice in water (5 g/4 ml water) using a rotational prophylaxis brush mounted on a low-speed handpiece at 2000 rpm (revolutions per minute) for 15 seconds. They were then rinsed with water for 60 seconds and dried by blotting with a cotton pellet.

In group D, following the application of aluminum chloride as explained in the control group, dentin conditioner (GC, Tokyo, Japan) was applied to the surfaces for 20 seconds using a cotton pellet, rinsed with water for 60 seconds, and dried by blotting with a cotton pellet.

In group E, following the application of aluminum chloride as explained in the control group, tooth surfaces were cleaned using a syringe containing 5 ml of 2% sodium hypochlorite (NaOCl) for one minute, rinsed with water for 60 seconds, and dried by blotting with a cotton pellet.

In group F, following the application of aluminum chloride as explained in the control group, tooth surfaces were cleaned using a syringe containing 5 ml of 2% CHX solution for one minute, rinsed with water for 60 seconds, and dried by blotting with a cotton pellet.

In all groups, the surfaces were etched with 37% phosphoric acid (3M ESPE, St. Paul, MN, USA) for 15 seconds and rinsed for 30 seconds. Excess water was removed using a cotton pellet such that the surface remained moist. Scotchbond Multi-Purpose (3M ESPE, St. Paul, MN, USA) primer was then applied to the surface for 15 seconds and dried with gentle air spray for 5 seconds. The bonding agent was then applied to the surface and light-cured for 20 seconds with a light-emitting diode (LED) unit (1600 mW/cm<sup>2</sup>; Demetron, Kerr, USA). Composite cylinders were then fabricated on the surfaces using Tygon tubes (Norton Performance Plastics Corp., Akron, OH, USA) with an internal diameter of 0.9 mm and a height of 2 mm. The Z250 composite (3M ESPE, St. Paul, MN, USA) was applied into the tubes in two increments of 1 mm thickness, and then, each increment was light-cured with the LED light-curing unit for 20 seconds. Then, the specimens were stored in distilled water at 37°C for 24 hours.

The samples were then subjected to thermocycling for 10,000 cycles between 5-55°C with 30 seconds of dwell time and 30 seconds of transfer time [11]. The microshear bond strength (SBS) was then measured using a universal testing machine (Z020; Zwick/Roell, Ulm, Germany) at a crosshead speed of 0.5 mm/minute. The microshear bond strength values were calculated in megapascal (MPa). The microshear bond strength data were analyzed using one-way analysis of variance (ANOVA). Pairwise comparisons were made using Tukey HSD (honestly significant difference) test. All statistical analyses were conducted at a significance level of  $P < 0.05$ .

## RESULTS

Table 1 shows the SBS of the study groups. The SBS was significantly different among the groups ( $P = 0.036$ ). The SBS was the highest in the groups that were cleaned with CHX ( $10.85 \pm 6.95$ ) and NaOCl ( $10.71 \pm 6.1$ ) and the lowest in the control group ( $5.72 \pm 3.34$ ) and the group that was cleaned with dentin conditioner ( $5.58 \pm 3.87$ ). The difference between other groups was not significant ( $P = 0.996$ ).

## DISCUSSION

This study assessed the effect of different cleansing protocols on the SBS of an etch-and-rinse adhesive to dentin contaminated with aluminum chloride hemostatic agent and showed significant differences among the cleansing methods used.

**Table 1.** Bond strength values (MPa) in the study groups

Groups	Treatments	Minimum	Maximum	Mean	Standard Deviation
Control	No treatment	1.78	10.8	5.72 <sup>a</sup>	3.34
A	Water spray	3.83	15.87	7.96 <sup>b</sup>	3.75
B	Aluminum oxide	5.64	19.97	9.42 <sup>b</sup>	3.67
C	Slurry of pumice in water	1.78	19.21	7.74 <sup>b</sup>	5.71
D	Dentin conditioner	1.78	13.18	5.58 <sup>a</sup>	3.87
E	Phosphoric acid	1.78	20.97	5.9 <sup>a</sup>	5.21
F	Sodium hypochlorite	4.95	22.62	10.71 <sup>c</sup>	6.1
G	Chlorhexidine	1.78	22.12	10.85 <sup>c</sup>	6.95

\*Bond strength values with the same letter are not significantly different (Tukey test)

Pucci et al [2] evaluated the effect of hemostatic agent application and use of cleaning agents on the bond strength of an etch-and-rinse adhesive to dentin and showed that CHX, when used as a cleanser, yielded a strong bond between dentin and composite resin, which was in agreement with our findings. Chaiyabutr and Kois [6] evaluated the effect of cleaning protocols for teeth contaminated with a hemostatic agent on the bond strength of self-adhesive luting cements to the tooth structure and showed that the application of aluminum oxide had the higher cleaning efficacy compared to water, which also confirmed our results. The same authors in another study indicated the superior efficacy of aluminum oxide compared to pumice paste [4]. Our study, however, did not find a significant difference in the bond strength of pumice paste and aluminum oxide groups. In the particle abrasion process, aluminum oxide powder hits the dentin surface, and the kinetic energy of the particles results in microscopic porosity in the surface [12]. Because of this impact, hemostatic contaminants may be removed from the surface, improving the bond strength. Chaiyabutr and Kois [4] also compared sandblasting with 27 $\mu$ m and 50 $\mu$ m aluminum oxide particles and found no significant difference in the bond strength of the two groups, which shows that the size of particles does not affect the bond strength. Therefore, in this study, the surfaces were sandblasted using aluminum oxide particles with a 27 $\mu$ m diameter.

Hemostatic agents have an acidic pH in the range of 0.7 to 3 and can remove the smear layer and cause demineralization of enamel and dentin [11]. They can also affect the quality of the hybrid layer [13]. Thus, the use of hemostatic agents without proper cleaning negatively affects bond strength [14]. Ajami et al [3] showed that a five-minute water rinse with high pressure increases the bond strength. Water rinse might have physically removed the unbound residue of the hemostatic agent, and the monomer infiltration might have improved, which is similar to our findings, but the duration of water rinsing (5 minutes) in their study is not

clinically acceptable [3].

Aluminum chloride hemostatic agent is effective for bleeding control. It has minimal side effects and is commonly used by dental clinicians. Moreover, it does not cause discoloration, as does the ferric sulfate [15]. Thus, aluminum chloride was evaluated as a hemostatic agent in the present study. The use of aluminum chloride reduces the bond strength of adhesives to dentin [4]. When aluminum chloride is applied on the tooth surface, the calcium in the hydroxyapatite is replaced by aluminum and results in the formation of Al(OH)2H2PO4. This might increase the resistance of the dentin surface to acid-etching. This phenomenon decreases monomer infiltration into the dentin and causes a decrease in the bond strength [16]. This may also be attributed to the deposition of unbound aluminum on the dentin surface [3].

The depth of dentin is an important factor affecting the bond strength because deeper dentin has a higher number and diameter of dentinal tubules, and therefore, the quality of resin tags would be different compared to that in the superficial dentin [17,18]. Therefore, to minimize the effect of this confounding factor, we tried our best to expose the mid-coronal dentin of all tested teeth for standardization.

In the present study, thermocycling was performed to better simulate the clinical setting and increase the generalizability of the results to the clinical condition. According to Holderegger et al [19], thermocycling affects the bonding durability to dentin. To simulate one year of aging in an oral environment, thermocycling of 10,000 cycles (5-55°C) was selected for our study.

The current study revealed that the elimination of hemostatic agents, except for GC conditioner, improved the bond strength, and CHX yielded the best result in this respect. Chlorhexidine is a cationic detergent with high antimicrobial activity. Because of its cleansing properties, it could remove residues of hemostatics and enhance the bond strength [2]. It also inhibits the activity of matrix metalloproteinases and decreases the dissolution of collagen fibers in an aqueous environment [2]. According to Pucci et al [2],

the application of CHX following the use of hemostatic agents significantly increases the bond strength of the tooth structure to composite resin. This finding was also confirmed by our results. However, Sharafeddin and Farhadpour [20] revealed that the use of CHX after the removal of the hemostatic agent reduced the bond strength to dentin. The difference in the results may be attributed to the different bonding agents used. There are different opinions about the proper step for using CHX. Some researchers have used CHX after etching to inhibit collagenolytic activity [21]. Some authors have used it before etching to clean the dentin surface [22]. According to Campos et al [23], there is no significant difference regarding the step in which CHX is applied. We used CHX before etching to remove the remnants of the hemostatic agent.

In the current study, cleaning the contaminated tooth surfaces with NaOCl also improved the bond strength values. Sodium hypochlorite (NaOCl) is used for root canal irrigation. It is a halogenated agent with antimicrobial action [24] and can remove the organic component of dentin due to its proteolytic action [25]. The latter action may lead to the removal of hemostatic precipitation and bond strength enhancement. Sodium hypochlorite also increases wettability [26], which could be a benefit in the case of bonding.

Ajami et al [3] compared the efficacy of three methods of eliminating the hemostatic agent from the tooth structure and indicated that phosphoric acid, compared to water, resulted in the better elimination of the hemostatic agent and increased the bond strength of self-etch adhesives. They attributed this finding to the replacement of  $\text{Al}(\text{OH})_2\text{H}_2\text{PO}_4$  with  $\text{AlPO}_4$ , which increases the penetration and subsequent interlocking of the bonding agent into the tooth structure [3]. Their results were different from ours, which may be due to different kinds of bonding agents.

Over-etching probably weakened the bond strength of samples decontaminated with dentin conditioner, which is based on the polyacrylic acid, compared to the use of water,

since we used a 3-step etch-and-rinse bonding agent, which also contains phosphoric acid etchant. Over acid-etching causes demineralization and degrades the collagen network [27].

The type of bonding agent affects bond strength [28]. We used Scotchbond Multi-Purpose, 3-step etch-and-rinse bonding agent because evidence shows that this bonding agent provides gap-free margins and yields a high bond strength. Moreover, etch-and-rinse bonding agents generally have higher shear bond strength than self-etch bonding agents [28].

This study had an in-vitro design, and in-vitro studies cannot perfectly simulate the oral clinical conditions. Thus, the generalization of the results to the clinical setting must be done with caution. Future studies are required to assess the effect of various cleansing modalities on the bond strength of self-etch bonding agents after the application of different hemostatic agents. In addition, microleakage of such restorations should be evaluated in future studies.

## CONCLUSION

After dentin surface contamination with ViscoStat Clear containing aluminum chloride, CHX and NaOCl yielded the highest bond strength among the tested cleansing protocols.

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## CONFLICT OF INTEREST STATEMENT

None declared.

## REFERENCES

1. Bernades Kde O, Hilgert LA, Ribeiro AP, Garcia FC, Pereira PN. The influence of hemostatic agents on dentin and enamel surfaces and dental bonding: a systematic review. *J Am Dent Assoc.* 2014 Nov;145(11):1120-8.

2. Pucci CR, Araújo RM, Lacerda AJ, Souza MA, Huhtala MF, Feitosa FA. Effects of Contamination by Hemostatic Agents and Use of Cleaning Agent on Etch-and-Rinse Dentin Bond Strength. *Braz Dent J*. 2016 Dec;27(6):688-92.
3. Ajami AA, Kahnamoii MA, Kimyai S, Oskoe SS, Pournaghi-Azar F, Bahari M, et al. Effect of three different contamination removal methods on bond strength of a self-etching adhesive to dentin contaminated with an aluminum chloride hemostatic agent. *J Contemp Dent Pract*. 2013 Jan 1;14(1):26.
4. Chaibabutr Y, Kois JC. The effect of tooth-preparation cleansing protocol on the bond strength of self-adhesive resin cement to dentin contaminated with a hemostatic agent. *Oper Dent*. 2011 Jan-Feb;36(1):18-26.
5. Tuncer D, Basaran S, Halacoglu DM, Yamanel K, Celik C, Arhun N. Effect of hemostatic agent application on the shear bond strength of contemporary/multi-mode adhesive systems. *Oral Health Dent Manag*. 2014 Mar;13(1):103-6.
6. Chaibabutr Y, Kois JC. The effect of tooth-preparation cleansing protocol on the bond strength of self-adhesive resin cement to dentin contaminated with a hemostatic agent. *Oper Dent*. 2011 Jan-Feb;36(1):18-26.
7. Arslan S, ERTAS H, Zorba YO. Influence of Ankaferd Blood Stopper on shear bond strength of bonding systems. *Dent Mater*. 2012 Mar;31(2):226-31.
8. Araújo IS, Prado CJD, Raposo LHA, Soares CJ, Zanatta RF, Torres CRG, et al. Influence of Hemostatic Solution on Bond Strength and Physicochemical Properties of Resin Cement. *Braz Dent J*. 2017 Sep-Oct;28(5):624-631.
9. Trakyalı G, Oztoprak MO. Plant extract ankaferd blood stopper effect on bond strength. *Angle Orthod*. 2010 May;80(3):570-4.
10. Khoroushi M, Hosseini-Shirazi M, Farahbod F, Keshani F. Composite resin bond strength to caries-affected dentin contaminated with 3 different hemostatic agents. *Gen Dent*. 2016 Jul-Aug;64(4):e11-5.
11. Moosavi H, Mohammadipour HS, Ghavamnasiri M, Alizadeh S. Effect of Bleaching and Thermocycling on Resin-Enamel Bond Strength. *Int J Biomater*. 2015;2015:921425.
12. Gray GB, Carey GP, Jagger DC. An in vitro investigation of a comparison of bond strengths of composite to etched and air-abraded human enamel surfaces. *J Prosthodont*. 2006 Jan-Feb;15(1):2-8.
13. Güngör AY, Alkis H, Turkkahraman H. Effects of contamination by either blood or a hemostatic agent on the shear bond strength of orthodontic buttons. *Korean J Orthod*. 2013 Apr;43(2):96-100.
14. Tarighi P, Khoroushi M. A review on common chemical hemostatic agents in restorative dentistry. *Dent Res J (Isfahan)*. 2014;11(4):423-8.
15. Nouri S, Sharif MR, Panahi Y, Ghanei M, Jamali B. Efficacy and safety of aluminum chloride in controlling external hemorrhage: an animal model study. *Iran Red Crescent Med J*. 2015 Mar 20;17(3):e19714.
16. Xu X, Chen Q, Lederer A, Bernau C, Lai G, Kaisarly D, Dent DM. Shear bond strength of two adhesives to bovine dentin contaminated with various astringents. *Am J Dent*. 2015 Aug;28(4):229-34.
17. Ebrahimi SF, Shadman N, Abrishami A. Effect of ferric sulfate contamination on the bonding effectiveness of etch-and-rinse and self-etch adhesives to superficial dentin. *J Conserv Dent*. 2013 Mar;16(2):126-30.
18. Sahin O, Dede DÖ, Köroğlu A, Özgüven Y, Doğan DÖ. Effect of irradiation on the shear bond strength of self-adhesive luting cement in different preparation depths. *Niger J Clin Pract*. 2017 Aug;20(8):924-929.
19. Holderegger C, Sailer I, Schuhmacher C, Schläpfer R, Hämmerle C, Fischer J. Shear bond strength of resin cements to human dentin. *Dent Mater*. 2008 Jul;24(7):944-50.
20. Sharafeddin F, Farhadpour H. Evaluation of Shear Bond Strength of Total- and Self-etching Adhesive Systems after Application of Chlorhexidine to Dentin Contaminated with a Hemostatic Agent. *J Dent (Shiraz)*. 2015 Sep;16(3):175-81.
21. Alaghehmad H, Mansouri E, Esmaili B, Bijani A, Nejadkarimi S, Rahchamani M. Effect of 0.12% chlorhexidine and zinc nanoparticles on the microshear bond strength of dentin with a fifth-generation adhesive. *Eur J Dent*. 2018 Jan-Mar;12(1):105-110.
22. Flury S, Peutzfeldt A, Schmidlin PR, Lussi A. Exposed Dentin: Influence of Cleaning Procedures and Simulated Pulpal Pressure on Bond Strength of a Universal Adhesive System. *PLoS One*. 2017 Jan 12;12(1):e0169680.
23. Campos EA, Correr GM, Leonardi DP, Pizzatto E, Morais EC. Influence of chlorhexidine concentration on microtensile bond strength of contemporary adhesive systems. *Braz Oral Res*. 2009 Jul-Sep;23(3):340-5.
24. Del Carpio-Perochena A, Monteiro Bramante C, Hungaro Duarte M, Bombarda de Andrade F, Zardin Graeff M, et al. Effect of Temperature, Concentration and Contact Time of Sodium Hypochlorite on the Treatment and Revitalization of Oral Biofilms. *J Dent Res Dent Clin Dent Prospects*. 2015 Fall;9(4):209-15.
25. Taneja S, Mishra N, Malik S. Comparative

evaluation of human pulp tissue dissolution by different concentrations of chlorine dioxide, calcium hypochlorite and sodium hypochlorite: An in vitro study. *J Conserv Dent*. 2014 Nov;17(6):541-5.

26. Abuhaimed TS, Abou Neel EA. Sodium Hypochlorite Irrigation and Its Effect on Bond Strength to Dentin. *Biomed Res Int*. 2017;2017:1930360.

27. Ryou H, Turco G, Breschi L, Tay FR, Pashley DH, Arola D. On the stiffness of demineralized dentin matrices. *Dent Mater*. 2016 Feb;32(2):161-70.

28. Favarão J, Zanini MM, Coelho Sinhoreti MA, Flores Abuna G, Girelli Júnior C. Immediate and 6-month Bond Strengths of Different Adhesives in the Oral Environment. *J Adhes Dent*. 2017 Dec 18:475-481.