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Bond Strengths of Two Adhesive Systems to Dentin Contaminated with a Hemostatic Agent

W Kuphasuk • C Harnirattisai
P Senawongse • J Tagami

Clinical Relevance

A self-etching adhesive exhibited significantly lower bond strength to dentin contaminated with 25% aluminum chloride solution compared to normal dentin, but a total-etching adhesive exhibited no difference in bond strength to either contaminated or normal dentin. Longer primer application of the self-etching adhesive significantly increased the dentin bond strength of the contaminated group.

SUMMARY

This study evaluated the bond strength of a total-etch and a self-etch adhesive to dentin contaminated with a hemostatic agent containing aluminum chloride ($AlCl_3$). Eighteen occlusal dentin discs were prepared from human molars. Each disc was ground and sectioned into two halves, one for normal dentin and the other for contaminated dentin. The specimens of both normal and contaminated dentin were randomly divided into three groups and treated with the following materials: 1) Excite (EX); 2) Clearfil SE Bond with 20-second primer application time (CB 20) and 3) Clearfil SE Bond with 40-second primer application time (CB 40). The microshear bond strength specimens were prepared using the resin composite Clearfil APX. The bond strengths were evaluated on a universal testing machine. Statistical analysis was performed at α=0.05. The surface micromorphology and aluminum content of the different dentin conditions were also examined. In EX, no significant difference was found between the bond strengths of normal dentin and contaminated dentin. The bond strength of CB20 to contaminated dentin was significantly lower than that to normal dentin. The

*Watcharaporn Kuphasuk, DDS, MSD, director of Graduate Operative Dentistry, Department of Operative Dentistry, Faculty of Dentistry, Mahidol University, Bangkok, Thailand

Choltacha Harnirattisai, BSc, DDS, PhD, associate professor, Department of Operative Dentistry, Faculty of Dentistry, Mahidol University, Bangkok, Thailand

Pisol Senawongse, DDS, MSc, PhD, lecturer, Department of Operative Dentistry, Faculty of Dentistry, Mahidol University, Bangkok, Thailand

Junji Tagami, DDS, PhD, professor and chairman, Cariology and Operative Dentistry, Department of Restorative Sciences, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan

*Reprint request: 6 Yothi St, Rachathewi, Bangkok 10400, Thailand; e-mail: dtwkp@mahidol.ac.th

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extension of primer application time from 20 to 40 seconds significantly increased the bond strength of CB to contaminated dentin.

INTRODUCTION

Moisture and blood contamination have a detrimental effect on bond strength between adhesives and tooth structures.[1-3] As a result, the use of a rubber dam is mandatory for all adhesive restorations.[4] In general practice, however, operators do not routinely work with a rubber dam, instead, other moisture control techniques are used. In some clinical situations, such as the gingival area, blood and sulcular fluid frequently appear as a result of gingival trauma from tooth preparation or gingival inflammation. Currently, in that condition, dry operative fields can be obtained after the application of hemostatic agents to control bleeding and decrease gingival fluid. Examples of these materials are aluminum chloride, aluminum sulfate and ferric sulfate. Previous studies have demonstrated that these hemostatic agents are highly acidic and their pH varies from 0.7-3.0.[5-6] Aluminum chloride ($AlCl_3$), with a concentration between 20%-25%, is a commonly used hemostatic agent.[7] It has been shown that dentin surfaces treated with 21.3% $AlCl_3$ exhibit various degrees of demineralization. Complete smear layer removal with some dentin demineralization can be observed after applying this agent for five minutes.[6]

Currently, adhesive systems can be classified into two groups, total-etching and self-etching systems. Since some effects of the smear layer to the adhesion of self-etching adhesive have been reported,[8] smear layer removal by hemostatic agents could affect the bonding mechanism of this adhesive system. It has been shown that the bond strength of a self-etching adhesive to dentin contaminated with ferric sulfate or $AlCl_3$ dramatically decreased, compared to the normal dentin group.[9]

One of the problems that occurs in bond testing is fracture of the specimens within the materials, not at the interface. Micro-tests, including a microtensile and a microshear bond test, have been developed to improve their efficiency.[10-12] This has resulted in an increase in specimens fracturing at the interface. Therefore, the bond strengths obtained from these tests should be more reliable and represent the true bond strength between materials. Also, the microshear bond test has some advantages, such as ease of specimen preparation and reliable results with a narrow standard deviation.[11-12]

This study evaluated the microshear bond strengths of a total-etch and a self-etch adhesive to human dentin contaminated with a hemostatic agent containing $AlCl_3$.

METHODS AND MATERIALS

Eighteen 2 mm-thick dentin discs were prepared by perpendicular sectioning to the long axis of extracted caries-free human molars using a low-speed saw, under copious water spray (Isomet, Buehler, IL, USA). The dentin surfaces were then hand ground with 600-grit SiC paper under running water and hemi-sectioned, resulting in 18 pairs of dentin semi-discs. Next, the pairs of semi-discs were randomly assigned to three groups of six pairs each. For each group, the six pairs of semi-discs were separated and subdivided into control and contaminated subgroups. The diagram of specimen preparation is shown in Figure 1 and the composition of the materials used in this study is shown in Table 1.

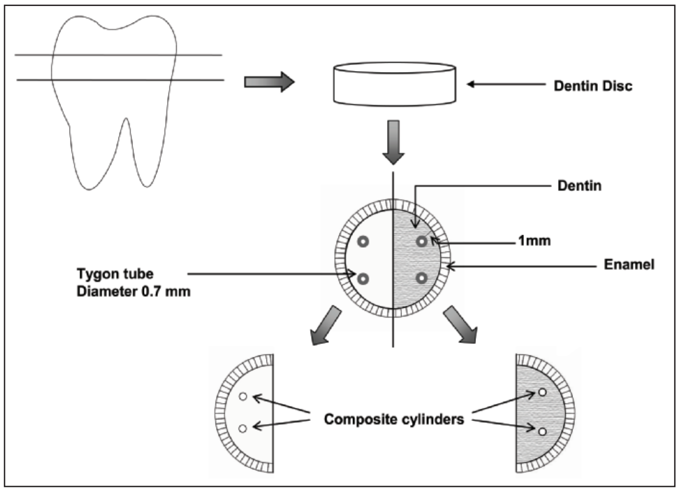

Figure 1. *Diagram illustrating bonding procedures for microshear bond strength test.*

The treatment protocol for each group was as follows: for Group 1 Excite (EX), the dentin surface of each semi-disc in the control subgroup was dried with oil-free air to remove excess water. In the contaminated subgroup, further dentin surface treatment was performed. The hemostatic agent Racestyptine (Septodont, Cedex, France) was applied to the dentin surfaces for two minutes, then the dentin was rinsed with water spray for 30 seconds and dried with oil-free air. Consequently, the dentin surfaces of both the control and the contaminated groups were etched with 37% phosphoric acid for 15 seconds and thoroughly rinsed using water spray. Excess water was blot-dried from the surface with lint-free paper (Kimwipes, Kimberly Clark Corp, Roswell, GA, USA) to achieve moist dentin. The adhesive Excite was used according to the manufacturer's instructions by applying the adhesive resin onto the dentin surface for 15 seconds, then drying with oil-free air for five seconds. The irises that were cut from micro bore tygon tubing (TYG-030, Small Parts Inc, Miami Lakes, FL, USA) with an internal diameter and height of approximately 0.75 and 0.50 mm, respectively, were then positioned at two locations on each dentin semi-disc, 1 mm from the dentino-enamel junction. Light polymerization was performed for 10 seconds with a light-curing unit

Table 1: *Materials Used in This Study*

Material	Composition	Batch #	Manufacturer
Racestyptine	25% m/V hexahydrate aluminum chloride, oxyquinol, hydroalcoholic excipient	M1 115	Septodont, Cedex, France
Clearfil SE Bond	*Primer*: HEMA, MDP, Hydrophilic dimethacrylate, water, ethanol, dl-camphorquinone, N,N-Diethanol-p-toluidine *Adhesive*: HEMA, MDP, Bis-GMA, Hydrophilic dimethacrylate, dl-camphorquinone, N,N-Diethanol-p-toluidine, silanated colloidal silica	00443 A 00609 A	Kuraray, Osaka, Japan
Excite	*Etchant*: 37% phosphoric acid *Adhesive*: Dimethacrylate, alcohol, phisphonic acid acrylate, HEMA, SiO_2, initiators, stabilizers	F 40503 F 63821	Ivoclar Vivadent, Schaan, Liechtenstein
Clearfil APX	BisGMA, TEGDMA, barium glass, colloidal silica	01028 A	Kuraray, Osaka, Japan

(Curing Light XL 3000, 3M ESPE, St Paul, MN, USA). A hybrid resin composite, Clearfil APX shade A2, was used to fill in the tubing and was light-cured for 40 seconds. The tubing was then removed from the composite cylinder by longitudinal cutting with a razor blade. This resulted in 12 composite cylinders for this adhesive, each in the control and contaminated groups. For Group 2 Clearfil SE Bond, 20 second primer application (CB 20), after the dentin surfaces were prepared for the control and contaminated subgroups in the same manner as in Group 1, Clearfil SE Bond was used according to the manufacturer's instructions. The primer was applied to the dentin surfaces with agitation, left for 20 seconds, then dried with oil-free air for five seconds. The adhesive resin was then applied to the primed surfaces. Next, the composite cylinders were prepared in the same manner as in Group 1. For Group 3 Clearfil SE Bond, 40-second primer application (CB 40), only the contaminated subgroup was performed. The specimen preparation and preparation for the microshear bond test were performed as in Group 2, except that the primer application time was extended from 20 to 40 seconds. Therefore, only 12 composite cylinders in the contaminated group were prepared. After storage in distilled water at 37°C for 24 hours, all specimens were inspected under an optical microscope (30x). The specimens with defects, such as interfacial gap defect and bubble inclusion, were excluded and replaced.

The microshear bond test was performed on the microshear bond test apparatus (Bencor-Multi-T, Danville Engineering Co, San Ramon, CA, USA) attached to a universal testing machine (EZ-test 500 N, Shimazu Co, Kyoto, Japan) as described by Shimada and others.[11] The dentin disc was placed on the apparatus with a cyanoacrylate adhesive (Zapit, Dental Venture of America, Corona, CA, USA). A thin wire, 0.2 mm in diameter, was looped around the small resin composite cylinder. This procedure makes the lower half of the cylinder contact the wire, which is gently held flush against the resin-dentin interface. The resin cylinder and the center of the load cell were aligned as straight as possible (Figure 2). A shear force was applied to each specimen at a crosshead speed of 1 mm/minute until fracture. Two-way ANOVA and multiple comparisons at $p<.05$ were used to analyze the data.

Morphological changes of the normal dentin surface after grinding, dentin contamination with a hemostatic agent and both dentin conditions after etching with phosphoric acid and self-etching primer application for 20 and 40 seconds were observed using a scanning electron microscope (JSM-5310V, JEOL Ltd, Tokyo, Japan). The specimens were observed and analyzed for aluminum content using an energy dispersive spectrometer (EDS, Oxford ISIS Pentafet Link Model 6647, High WyCombe, England) operated at 20 KV.

A pH meter (Twin pH, Horiba, Tokyo, Japan) was used to determine the pH of the hemostatic agent.

RESULTS

The pH of the hemostatic agent, Racestyptine, consisting of 25% $AlCl_3$, was 0.8. Table 2 shows the microshear bond strengths of the adhesives used in this study to normal and contaminated dentin. The microshear bond strength of Excite adhesive to normal dentin and contaminated dentin were 18.42 ± 2.28 and 22.49 ± 5.89 MPa, respectively. No statistically significant difference between these two groups was exhibited ($p>.05$). The microshear bond strength of the self-etching adhesive Clearfil SE Bond to normal dentin was 36.59 ± 5.94 MPa, which was significantly higher than the microshear bond strength of this adhesive to contaminated dentin (CB20), 19.35 ± 6.05 MPa ($p<.05$). The microshear bond strength of Clearfil SE Bond to contaminated dentin, when the primer application time was extended to 40 seconds, 29.09 ± 6.93 MPa, was significantly higher than that of the contaminated group with a 20 second primer application ($p<.05$). Nevertheless, the bond strength of the 40 second primer application group was still significantly lower than that of the control group ($p<.05$), which was the highest bond strength obtained in this experiment.

Scanning electron micrographs of the dentin surface in the control group revealed that the thick smear layer was left intact on the surfaces and the dentinal tubules could not be seen (Figure 3). In the contaminated group, noticeable etching effects were observed. The smear layer was partially removed and the dentinal tubule opening was located. However, the smear plug still occluded the tubule orifices (Figure 4). The surfaces of normal and contaminated dentin after phosphoric acid etching were similar, with the absence of the smear layer and peritubular dentin, and the clearly visible patent opening of the dentinal tubules were exhibited (Figures 5 and 6). After treatment with SE primer for 20 seconds, normal dentin revealed clear surfaces without smear layers and open tubules with the remaining peritubular dentin (Figure 7), while the contaminated surface treated with SE primer for 20 seconds showed surfaces without smear layers, with some tubules still occluded (Figure 8). With the 40 second SE primer application, the contaminated dentin surface exhibited a more pronounced etching effect, with the surface of the smear layer depleted and more widely open dentinal tubules without peritubular dentin. Well-defined peritubular collagen fibers could be observed inside the tubules (Figure 9).

EDS analysis showed more aluminum content in the groups of contaminated dentin and contaminated dentin treated with SE primers at both 20 and 40 seconds (3.22%-4.76%Al) compared with normal dentin (0.49%Al) and contaminated dentin treated with phosphoric acid (0.46%Al).

Table 2: *Microshear Bond Strengths (MPa) of a Total-etching Adhesive, Excite and a Self-etching Adhesive, Clearfil SE Bond, to Normal Dentin and Contaminated-dentin*

Groups	Normal Dentin	Contaminated Dentin
Excite (EX)	18.42 ± 2.28[c]	22.49 ± 5.89[c]
Clearfil SE Bond: 20 seconds primer (CB20)	36.59 ± 5.94[a]	19.35 ± 6.05[c]
Clearfil SE Bond: 40 seconds primer (CB40)	--	29.09 ± 6.93[b]

Groups with the same superscript are not statistically different (p>0.05).

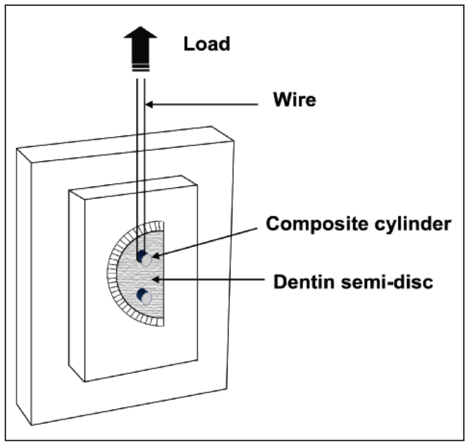

Figure 2. *Schematic of the microshear bond test.*

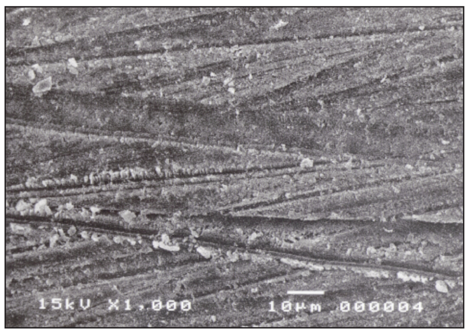

Figure 3. *Scanning electron micrograph of normal dentin after grinding with 600 grit SiC paper. Thick smear layer covered the dentin surface. No dentinal tubule opening was visible (1000x).*

DISCUSSION

In this study, specimens in the contaminated group and control group were prepared on the same dentin disks. Therefore, variables from different dentin substrates, such as age of the tooth and storage condition, could be excluded. Since dentin depth is one factor affecting the dentin bond strength of adhesives,[13-14] the dentin level was controlled in this study by fabricating resin composite cylinders at the same distance, 2 mm from the dentino-enamel junction.

Bond strength of the total-etch system in this study was significantly lower than that of the self-etching system. Results appear to be similar to the 2006 study by De Munck and others.[15] The low bond strength of a total-etching adhesive (Scotchbond 1), 11.9 MPa, compared with that of a self-etching system (Clearfil SE Bond), 41.3 MPa was also demonstrated. The explanation may be that the total-etching system is very technique sensitive. The dentin should be properly moist. Moreover, the dentin etched by an acid may be too deep to be penetrated by the adhesive. This results in nanoleakage, which possibly occurs with the total-etching system. Dentin bond strength in the microtest, microtensile or microshear bond strength test of Clearfil SE Bond was frequently found to be a high value, 32.9 MPa[16] and 39.81 MPa.[17] In contrast, studies showed the wide range of microtensile bond strength of Excite to be 6.03 MPa[18] and 40.8 MPa.[19]

The hemostatic agent containing 25% $AlCl_3$, Racestyptine, was selected as a representative agent, because it is effective in controlling bleeding and is frequently used in clinical practice. The two-minute con-

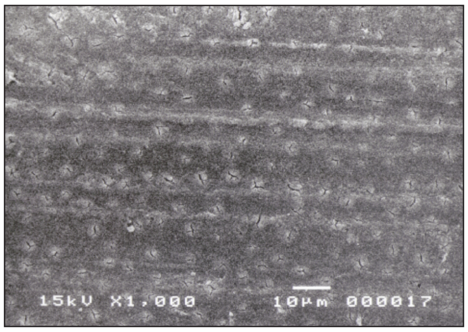

Figure 4. *Scanning electron micrograph of dentin contaminated with 25% aluminum chloride for two minutes. The smear layer was partially removed and the dentinal tubule orifices can be localized (1000x).*

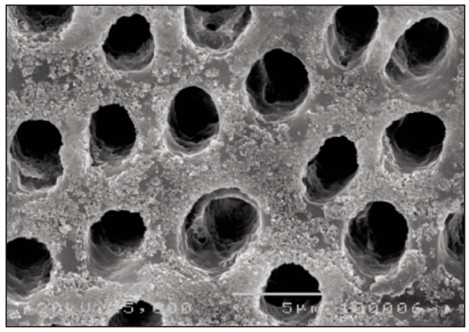

Figure 5. *SEM observations demonstrating the absence of the smear layer, peritubular dentin and patent tubule openings of normal dentin after etching with 37% H_3PO_4 (5000x).*

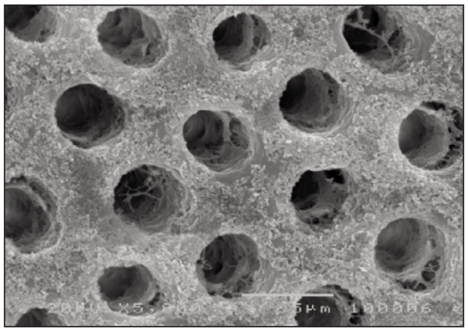

Figure 6. *Contaminated dentin appears similar to normal dentin after etching with 37% H_3PO_4, as shown in Figure 5 (5000x).*

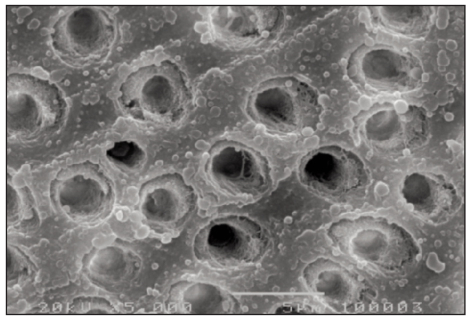

Figure 7. *Normal dentin after Clearfil SE Bond primer application for 20 seconds. The smear layer is completely removed; dentinal tubules with peritubular dentin are observed (5000x).*

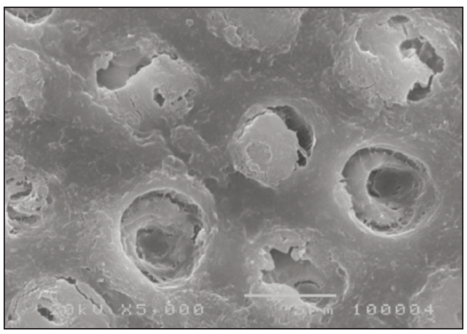

Figure 8. *Contaminated dentin after Clearfil SE Bond primer application for 20 seconds reveals no smear layer, but some tubules are occluded with smear plug (5000x).*

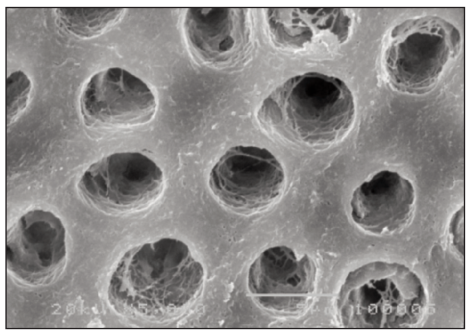

Figure 9. *Contaminated dentin after Clearfil SE Bond primer application for 40 seconds. More aggressive etching pattern is detected, compared with Figure 8. Complete smear layer removed; wide open dentinal tubules without peritubular dentin are exhibited (5000x).*

tamination time was chosen, as it is the average application time when this solution is applied onto soft tissues to control bleeding before restoration is initiated. The results indicated that the $AlCl_3$ solution had some demineralizing effect on the dentin surface. However, the degree of demineralization was less than the previous study,[7] which showed an aggressive etching pattern with complete smear layer removal. An explanation might be the shorter contamination time in this study, two minutes, instead of five minutes, as in the previous study. The degree of dentin surface changes after exposure to 21.3% $AlCl_3$ solution, Hemodent, has been shown to depend on contamination time. Dentin exposed to 21.3% $AlCl_3$ solution for two minutes exhibited smear layer removal and partially occluded dentinal tubules, while dentin exposed to this solution for five minutes revealed a totally removed smear layer, including demineralized peritubular dentin. Nevertheless, at the 30-second and 2-minute exposure times, the affected dentin surfaces were similar.[7]

Although some demineralization of dentin contaminated with the $AlCl_3$ solution was exhibited in the current study, application of the self-etching primer on contaminated dentin did not enhance its demineralization effect. In contrast, after priming with the self-etching adhesive, the contaminated dentin showed a less etching effect compared to the control group, where the dentin was normal. In addition, the dentin bond strength of CB 20 on the contaminated group was dramatically decreased, compared to that of the control group. The same result was also reported in a previous investigation.[9]

It has been shown that enamel treated with $AlCl_3$ solution for 20 minutes could uptake aluminum (Al) from the solution, especially within the first 20 µm of enamel.[20] Moreover, this $AlCl_3$ treated enamel revealed inhibition of the demineralization process of hydroxyapatite (HAP), which was exposed to a demineralizing solution,[21-22] even though the Al concentration was as low as 0.1 µmol/l.[23] This mechanism has been explained by displacement of calcium in the HAP by Al, which results in the very insoluble $Al(OH)_2 H_2PO_4$ compound.[24] Because HAP is also the major part of dentin-like enamel, the influence of $AlCl_3$ solution on dentin could be similar to enamel.

Since the Clearfil SE primer has weak acidity, with the pH being approximately 2,[25] the demineralizing effect on dentin contaminated with $AlCl_3$ solution might be similarly inhibited. For self-etching adhesives, the dentin bonding mechanism is due to the exposed collagen network and smear layer modification by self-etching primer incorporated into resin adhesives. As a result, less dentin etching effect of the primer could result in bond strength decreases, as shown in this study. The results of EDS analysis confirmed that a higher aluminum content remained on the contaminated dentin surface following application of SE primer for either 20 or 40 seconds. Nevertheless, the 40-second primer application might be a proper method to use for Clearfil SE Bond when the dentin surface is contaminated with this hemostatic agent, since the bond strength in this group was significantly higher than that in the CB 20 group. The surface morphology of the CB 40 group showed more aggressiveness of the etching pattern. Extending the primer application time of the self-etching adhesive might enhance the etching effect of the primer and can result in higher dentin bond strength of this adhesive system.

However, for the total-etching adhesive used in this study (EX), the contamination of dentin with $AlCl_3$ solution did not have a detrimental effect on bond strength. The microshear bond strengths of the control and contaminated group were comparable. This might be due to the aggressive etching effect of phosphoric acid, with pH 0.5,[26] which simultaneously demineralized and removed all contaminants on the affected dentin surfaces. This was suggested by the fact that contaminated dentin and normal dentin, after phosphoric acid etching, revealed similar remaining aluminum content that was less than that of contaminated dentin and contaminated dentin treated with SE primer for both 20 and 40 seconds. Moreover, the dentin etching patterns of the control and contaminated group of EX after acid etching were similar.

From the SEM of this study, the total-etching adhesive showed the complete smear layer and peritubular dentin removal after phosphoric acid etching; therefore, it could enhance fluid movement across the resin-dentin interface. In contrast, the self-etching system could result in less fluid movement due to a less aggressive etching pattern, resulting in superior dentin sealing compared with the total-etching system.[27] This might be the reason why the self-etching adhesives exhibited less incidence of post-op sensitivity.[28]

Currently, few studies have reported the effect of hemostatic agent on the bond strength of adhesives to tooth structures. The different composition of the hemostatic agents might affect tooth structure differently. Thus, future studies reporting on this aspect are needed. Given the results of this study, care should be taken when the hemostatic agent is used before application of self-etching adhesives. Extending the primer application time of the self-etching adhesive or using the total-etching systems might be appropriate in this situation.

CONCLUSIONS

When self-etching adhesive was used, dentin contaminated with the hemostatic agent Racestyptine, containing 25% aluminum chloride, had significantly lower bond strength compared to normal dentin. The hemostatic agent did not have any effect on dentin bond strength of the total-etching adhesive. These results are limited to the materials used in this study. Other materials might perform differently from these findings.

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