

Effect of dentin contamination with two hemostatic agents on bond strength of resin-modified glass ionomer cement with different conditioning

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This study evaluated the effect of two hemostatic agents on bond strength of resin-modified glass ionomer cement (RMGIC) to root dentin after using different pretreatment solutions compared to resin composite with a 2-step self-etch adhesive. Dentin specimens were either uncontaminated or contaminated with a ferric sulphate or aluminum chloride astringent, RMGIC was applied after conditioning with; Cavity Conditioner, Self Conditioner or Clearfil SE Primer, while a resin composite was bonded using Clearfil SE Bond. Microtensile bond strength (μ TBS) values and failure modes were determined. Two-way ANOVA revealed that hemostatic agents did not significantly affect the RMGIC bond strength with any conditioning material ($p > 0.05$). SE Primer pretreatment provided the highest μ TBS values among RMGIC groups. For the resin composite, ferric sulphate astringent significantly reduced μ TBS value ($p \leq 0.001$). Scanning electron micrographs of representative dentin surfaces contaminated with the hemostatic agents showed effects on smear layer removal and dentinal tubule occlusion.

Keywords: Hemostatic agents, Bond strength, Resin-modified glass ionomer cement, Dentin conditioning, Resin composite

INTRODUCTION

One of the clinical challenges that dentists face during restorative procedures, especially in the cervical area, is blood and gingival crevicular fluid contamination which may affect the quality of the bond between a restoration and tooth structure^{1,2}. Therefore, where possible, rubber dam placement is used to achieve optimal isolation, however, in some clinical situations rubber dam placement is not possible³.

Topical hemostatic agents have been developed and used as a method to achieve hemostasis before placement of a restoration. Among these hemostatic agents; aluminum chloride (AlCl_3) used at a concentration between 0–25% which achieves hemostasis by protein precipitation and constriction of blood vessels⁴, and 20% ferric sulfate ($\text{Fe}_2(\text{SO}_4)_3$) solutions which achieve hemostasis *via* chemical reaction of both the ferric and sulfate ions to agglutinate blood proteins and form plugs to occlude the capillary orifices⁵. Because clinicians may use hemostatic agents by soaking a gingival retraction cord before placement, or rubbing the hemostatic agent against the tissues before retraction cord application, contamination of the prepared cavity to be restored is unavoidable. Previous studies investigated the effect of this contamination on dentin as well as the bond strength to resin composite and resin cements showing that both AlCl_3 and $\text{Fe}_2(\text{SO}_4)_3$ resulted in a disturbance of the dentinal smear layer^{6,7}, demineralization of dentin⁸, and reduction in bond strength of resin composite and self-adhesive resin cements⁸⁻¹².

Resin-modified glass ionomer cement (RMGIC) is a restorative materials having less sensitivity to water balance and superior physical properties compared to conventional glass ionomer cements^{13,14}, with a fluoride releasing property¹⁵, chemical bond to dental tissue and adhesion in wet field¹⁶. This makes it a restorative choice for deep subgingival lesions, including the open and closed sandwich techniques^{17,18} and as a core material¹⁹. In these situations, contamination of the prepared cavity in root dentin with a hemostatic agent is more likely to happen.

RMGIC incorporates both chemical adhesion to the tooth structure by ionic interaction between the calcium in hydroxyapatite (HAp) of tooth structure and carboxyl groups of the RMGIC, in addition to a resin based micromechanical interlocking²⁰. Previous studies reported that using a conditioning agent is mandatory to enhance the bond of the RMGIC to prepared dentin^{21,22}, some other studies reported that dentin treatment with a self-etch adhesive system (SEA) containing primer and bonding agent was able to significantly enhance the bond of RMGIC^{16,23,24}, the role of the primer is to simultaneously etch and prime tooth substrate, enhancing the monomer penetration into the hydrophilic dentin and improving its wettability^{25,26}, and this may enhance the resin bonding mechanism of RMGIC to dentin. There is currently no information testing whether the use of a self-etch primer without bonding agent prior to RMGIC placement will provide good adhesion compared with that achieved by dentin conditioners. In addition, it still remains unclear if hemostatic agents will affect the

bond strength of RMGIC to dentin. Therefore, the aim of this study was to evaluate the effect of two hemostatic agents containing AlCl_3 or $\text{Fe}_2(\text{SO}_4)_3$ on bond strength of RMGIC to root dentin after using different conditioning solutions that includes the use of a self-etching primer from an enamel-dentin adhesive system.

MATERIALS AND METHODS

Materials used in this study

Two restorative materials were used; RMGIC (Fuji II LC capsule, GC, Tokyo, Japan), and resin composite (Clearfil APX, Kuraray Noritake Dental, Tokyo, Japan)

shade A2, two hemostatic agents; Viscostat Clear (Ultradent Products, South Jordan UT, USA), an AlCl_3 hemostatic agent, and Viscostat (Ultradent Products), a $\text{Fe}_2(\text{SO}_4)_3$ hemostatic agent, the conditioning solutions were; Cavity Conditioner (GC), Self Conditioner (GC), Clearfil SE primer (Kuraray Noritake Dental), or two-step self-etch adhesive Clearfil SE Bond (Kuraray Noritake Dental), their compositions and methods of application are presented in Table 1. Sixty extracted bovine teeth were collected, pulp tissues removed and the root surfaces cleaned using periodontal curettes, then a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) was used to separate the crown and the

Table 1 Restorative and conditioning materials used, manufacturer, batch no., composition and method of application

Material	Brand name and manufacturer	Code	Chemical composition	Method of application
Hemostatic agents	Viscostat Clear (Ultradent Products, South Jordan, UT, USA) (B#: BD6BT).	VSC	25% aluminum chloride	The gel applied on dentin surface for 5 min and rinsed with water for 20 s.
	Viscostat (Ultradent Product) (B#: BDB4S).	VS	20% ferric sulfate	The gel applied on dentin surface for 5 min and rinsed with water for 20 s.
Conditioning agents	Cavity Conditioner (GC, Tokyo, Japan) (B#:1508101).	CC	77% distilled water, 20% polyacrylic acid, 3% aluminum chloride hydrate	Applied to cavity surface for 10 s. Rinsed thoroughly with water and dried without desiccation.
	Self Conditioner (GC) (B#:1411111).	SC	20–30% HEMA, 5–10% 4-META, 30–35% distilled water, 28–40% ethanol	Applied to cavity surface and left undisturbed for 10 s. Air dried for 5 s.
	Clearfil SE primer (Kuraray Noritake Dental, Tokyo, Japan) (B#: 9H0109).	SEP	MDP, HEMA, hydrophilic aliphatic dimethacrylate, di-Camphorquinone, N-diethanol-p-toluidine, water	Primer applied to cavity surface with continuous rubbing for 20 s, Air dried for 5 s.
Self-etch adhesive system	Clearfil SE Bond (Kuraray Noritake Dental)	—	Primer: MDP, HEMA, hydrophilic aliphatic dimethacrylate, di-Camphorquinone, N-diethanol-p-toluidine, water (B#: 9H0109) Adhesive: MDP, Bis-GMA, HEMA, hydrophilic dimethacrylate, and microfiller (B#: 1R0296)	Primer applied to cavity surface with continuous rubbing for 20 s then air dried for 5 s. Bond applied to surface, spread uniformly with light air stream, and light cured for 10 s.
Restorative materials	Fuji II LC capsule shade A2. (GC) (B#: 1610137)	—	Fluoro-alumino-silicate glass, Polyacrylic acid, HEMA, urethane dimethacrylate, camphorquinone, water	Capsule mixed for 10 s and applied on dentin surface, then Light cured for 20 s.
	Clearfil AP-X shade A3 (Kuraray Noritake Dental, Japan) (B#: 440059)	—	Bis-GMA, TEGDMA, filler (Barium, SiO_2), dl Camphorquinone Catalysts Accelerators	Applied as one increment not exceeding 2 mm in thickness and light cured for 40 s.

Bis-GMA: bisphenol-A-diglycidylmethacrylate, HEMA: 2-hydroxyethylmethacrylate, MDP: 10-methacryloyloxydecyl dihydrogen phosphate, 4-META: 4-methacryloxyethyl trimellitate anhydride, TEGDMA: triethyleneglycol dimethacrylate

apical two-thirds of the root under water cooling, the remaining central/cervical root portion was sectioned along the root long axis and transversally to obtain two dentin blocks from cervical part of the root just apical to the cemento-enamel junction. Each root dentin block was embedded in epoxy resin (Epoxicure 2, Buehler) using a cylindrical mold. After setting of the resin the root surface was ground manually with #600-grit SiC paper under running water to expose a flat standardized dentin surface.

Specimen preparation

The specimens were divided according to the dentin surface treatment with hemostatic agents into untreated (control) or treated (contaminated) with one of two hemostatic agents, Viscostat Clear (VSC) or Viscostat (VS). their application time was five minutes^{6,7,10} followed by rinsing off with a vigorous water spray for 20 s, after this, for the RMGIC groups the surface was treated with either Cavity Conditioner (CC), Self Conditioner (SC), or Clearfil SE Primer (SEP), according to the manufacturer's instructions (Table 1). Fuji II LC capsule was mixed using a GC Capsule Mixer (CM-II, GC) for 10 s, applied on the dentin surface in a cylindrical mold of 2 mm thickness and light cured for 20 s using a quartz halogen light curing unit (Optilux 501, Kerr, Danbury, CT, USA) at 600 mW/cm².

For the resin composite groups, Clearfil SE Bond was applied according to the manufacturer's instructions (Table 1) on dentin contaminated with one of the hemostatic agents or on non-contaminated surface used as control (CR/control). Then the resin composite Clearfil APX was placed using the same cylindrical mold and light cured for 40 s. All the specimens were stored separately in distilled water at 37°C for 24 h.

Microtensile bond strength (μ TBS) test

After storage, each bonded specimen was sectioned longitudinally both buccal-lingually and mesiodistally

across the bonded interface to obtain multiple sticks of approximately 1 mm² for the μ TBS test. A total of 40 sticks obtained from five different teeth for each subgroup were tested. A digital caliper (Mitutoyo, Kawasaki, Japan) was used to check the cross-sectional area of each stick, which was then fixed to the test apparatus with a cyanoacrylate adhesive (Zapit, Dental Ventures of American, Anaheim Hills, CA, USA) and stressed in tension on a universal testing machine (EZ-Test, Shimadzu, Kyoto, Japan) at a crosshead speed of 1 mm/min until failure. The procedures of specimen preparation for the μ TBS test are shown briefly in Fig. 1.

Failure mode analysis

After debonding, specimens were fixed on a specimen holder with double-sided adhesive tape and sputter-coated for evaluation under SEM (S-4500, Hitachi, Tokyo, Japan) at 15 kV accelerating voltage. Failure modes were classified into the following categories; adhesive failure (A) (failure at the dentin-material interface), cohesive failure in restorative material (C), cohesive failure in dentin (D), and mixed failure (M), (partially adhesive and cohesive failure).

SEM observations of the contaminated dentin surfaces

To evaluate the effect of contamination with the hemostatic agents, dentin surfaces with/without hemostatic agent application were prepared. The specimens were then desiccated for 24 h, and gold sputter-coated for SEM observation (S-4500, Hitachi).

Statistical analysis

Normal distribution of data was assumed after Kolmogorov-Smirnov and Shapiro-Wilk tests. Two-way analysis of variance (ANOVA) was used to compare between different tested conditioning Materials and hemostatic Agent on mean μ TBS (MPa) followed by pairwise comparisons with Bonferroni correction. (SPSS V.23, SPSS, Chicago, IL, USA).

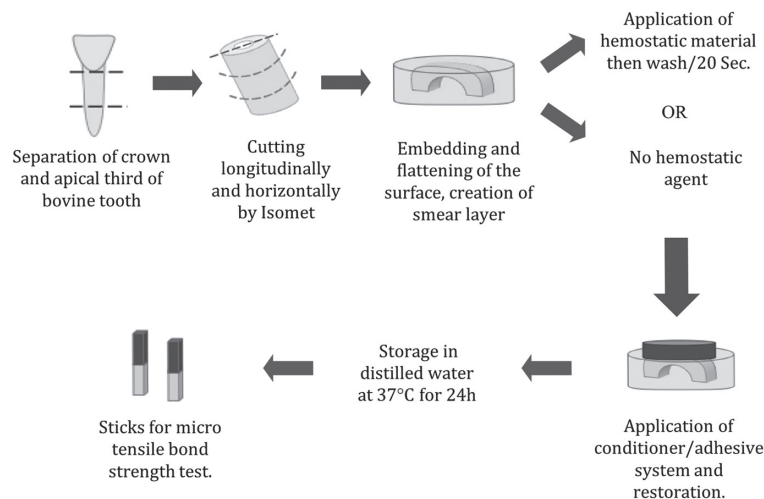


Fig. 1 Schematic showing specimen preparation for the μ TBS test.

RESULTS

Table 2 shows the mean μ TBS values of the RMGIC and resin composite to dentin contaminated with the hemostatic agents.

Two-way ANOVA showed that conditioning materials had a highly significant effect on mean μ TBS at $p \leq 0.001$, while the hemostatic agents showed non-significant effect on mean μ TBS at $p > 0.05$. Among RMGIC groups, SEP showed the highest bond strength values, followed by SC, then CC which showed the lowest μ TBS values for all conditioning agents with significant differences between each of them at $p \leq 0.001$.

Contamination with VSC or VS caused no significant change ($p > 0.05$) in bond strength among all RMGIC groups. For the resin composite groups, VSC caused no significant reduction in μ TBS, while VS contamination led to a significant reduction ($p \leq 0.001$). The μ TBS values of all the RMGIC groups were significantly lower than the resin composite groups.

Regarding the results of the failure mode analysis (Table 3), the mixed mode of failure (M) predominated for all groups, except for CC/VS, which was adhesive

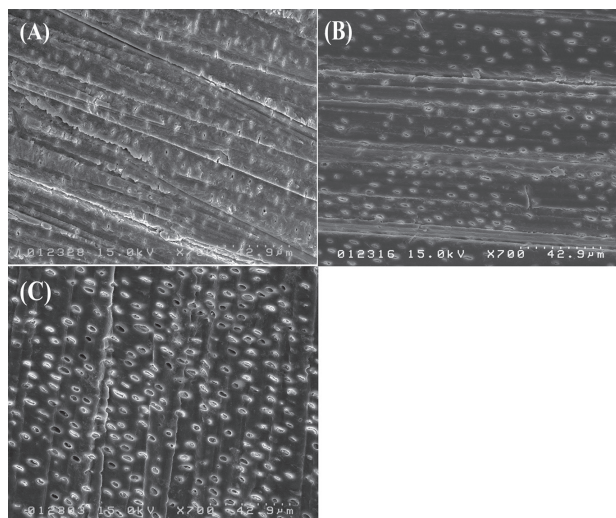


Fig. 2 SEM images of dentin surfaces after finishing with 600-grit SiC paper and (A) rinsing with water for 20 s, (B) application of Viscostat Clear for 5 min and rinsing with water for 20 s, (C) application of Viscostat for 5 min and rinsing with water for 20 s.

Table 2 Mean values and (SD) of μ TBS (MPa) of the tested groups

		Control	VSC	VS
RMGIC Fuji II LC	CC	17.1 (2.3) ^a	17.7 (2.8) ^a	16.1 (2.8) ^a
	SC	26.0 (4.6) ^b	24.6 (3.6) ^b	23.4 (3.2) ^b
	SEP	33.8 (5.8) ^c	30.8 (4.8) ^c	28.9 (4.3) ^c
AP-X composite resin	SE Bond	55.6 (5.8) ^e	51.9 (6.1) ^e	43.7 (7.1) ^d

Groups identified by similar small letters were not significantly different at $p > 0.05$.

CC: Cavity Conditioner, SC: Self Conditioner, SEP: Clearfil SE primer, VSC: Viscostat clear, VS: Viscostat

Table 3 Failure mode (in percent) of the debonded specimens of different experimental groups

Material	Conditioning	Hemostatic agent	A	M	C	D
RMGIC Fuji II LC	CC	control	35	40	25	0
		VSC	38	38	24	0
		VS	38	35	27	0
	SC	control	17	45	33	5
		VSC	25	45	30	0
		VS	30	43	27	0
	SEP	control	10	35	45	10
		VSC	9	40	39	12
		VS	14	43	38	5
AP-X composite resin	SE Bond	control	20	63	8	9
		VSC	18	63	9	10
		VS	24	65	5	6

A: adhesive failure, M: mixed failure, C: cohesive failure in restoration, D: cohesive failure in dentin

failure (A) and the SEP/control group which exhibited predominantly cohesive failure in restoration. It was noted also that the adhesive failure rate increased slightly among the Viscostat contaminated groups.

The morphological characteristics of the dentin surfaces after hemostatic agent contamination are shown in Fig. 2. In the control group (no treatment) the smear layer remained intact and thick, and dentin tubules could not be clearly identified (Fig. 2A). The hemostatic agent contaminated specimens showed noticeable etching effects of the dentin. For the AlCl_3 application group (Fig. 2B), most of the smear layer was removed exposing dentinal tubules, however, they remained occluded in most cases. For the $\text{Fe}_2(\text{SO}_4)_3$ group (Fig. 2C) the etching effect was more pronounced with the dentinal tubules remaining partially occluded.

DISCUSSION

AlCl_3 and $\text{Fe}_2(\text{SO}_4)_3$ containing hemostatic agents are among the most common topical hemostats in restorative dentistry. Current agents were in a gel form, which has a lower potential to spread over dentin surface than liquid ones, however, their viscous nature makes it harder to be removed from a dentin surface¹². The 5-min exposure time of the hemostatic agent was chosen, as long exposure times are not unusual especially with complex restorative procedures when there are multiple tooth preparations⁷.

The 2-step self-etch adhesive system and resin composite showed reduced μTBS values to dentin for both of the hemostatic agents. This result is consistent with those of the previous studies^{9,12,27}, although the application time of the hemostatic material was different in each study. It was shown previously^{6,7} that the acidic pH of the tested astringents resulted in smear layer removal, and dentin etching effects, hence confirmatory SEM radiographs of dentin surface treated with the current hemostatic agents were obtained. For the $\text{Fe}_2(\text{SO}_4)_3$, it is postulated that the changes to the dentin surface were responsible for this reduction. Previous studies showed that 5 min application of $\text{Fe}_2(\text{SO}_4)_3$ resulted in severe etching^{6,7,10}, which is consistent with the SEM micrographs from the current study, however, the dentinal tubules remained partly occluded. It was postulated that collagen or plasma proteins could have been coagulated by the $\text{Fe}_2(\text{SO}_4)_3$ ¹² that were unable to be cleansed or removed totally by the self-etch primer, thus, affecting the bond strength. For the AlCl_3 containing hemostatic agent, the reduction (which was non-significant) may be attributed to the higher Al content remaining on the contaminated dentin surface even after application of self-etch primer—as previously proven by energy dispersive spectrometer (EDS) analysis^{8,28}—resulting in displacement of calcium in the HAp by Al, forming an insoluble $\text{Al}(\text{OH})_2\text{H}_2\text{PO}_4$ compound which cannot be easily removed by the self-etching monomer, however, a previous study⁸ showed that this reduction was significant. Regarding the effect of the AlCl_3 on the dentin surface, it caused noticeable

dentin etching but not severe as the $\text{Fe}_2(\text{SO}_4)_3$ ⁷.

Cavity Conditioner is recommended by the manufacturer as the conditioning agent for Fuji II LC. It is composed of 20% polyacrylic acid and 3% aluminum chloride hydrate, its mode of action includes (1) cleaning the surface by removal of the smear layer (2) and a partial demineralization effect which increases the surface area for bonding by creating micro-porosities for micro-mechanical interlocking and formation of shallow hybrid layers that also contribute to the micro-mechanical bonding^{29,30}.

The bond strength of RMGIC can be enhanced by improving the resin bonding mechanism because of its resin component, as well as the quality of the hybrid layer. Self Conditioner was reported to increase the bond strength of RMGIC^{21,22}, it contains both 4-META, a functional monomer that chemically interacts with the HAp in dentin^{31,32}, and HEMA, which improves the dentin wettability and RMGIC spread and penetration into the dentin²⁶. When combined, these factors can improve the quality of the hybrid layer³³ which may be the major source of the enhanced bond strength²². Using SE primer only as pretreatment agent eliminates the need of bonding agent application or light curing, which is easier and more time saving compared to SEA. SE primer may have improved the bond strength in a similar fashion as SC, as both have a similar pH (SE primer 2.0 and SC 1.8). But the superior bond strength values with SE primer may be attributed to its MDP content as a functional monomer or its longer application time when the manufacturer recommendation was followed.

Dentin contamination with either of hemostatic agents used in this study did not significantly affect the bond strengths of the RMGIC groups, especially when Cavity Conditioner was used. The bonding of RMGIC to tooth structure is achieved *via* primary chemical bonding occurring through the formation of ionic bonds between the carboxyl groups of the polyalkenoic acid and calcium of HAp that remained around the exposed surface collagen, and micromechanical interlocking, achieved by infiltration of the organic tags of RMGIC components into a partially de-mineralized dentin surface²⁴. This two-fold bonding mechanism was not significantly affected after using the hemostatic agents. It has been previously reported that phosphoric acid etching improved the effect of self-etch adhesive in enhancing the bond strength of RMGIC²². Therefore, the etching effect of the hemostatic agents may have influenced the RMGIC bonding in the SC and SEP groups favorably. However, further investigation is required to better understand the exact reason for these results.

It should be mentioned that many previous experiments have used resin composite, unlike conventional glass ionomer cement, therefore it was preferred to be the positive control restorative material, because an expected result can be presumed from previous literature. In addition, a sort of similarity between the contamination effect of viscostat/viscostat clear and other $\text{Fe}_2(\text{SO}_4)_3$ / AlCl_3 astringents can be confirmed with the composite resin groups. The resin content in Fuji II

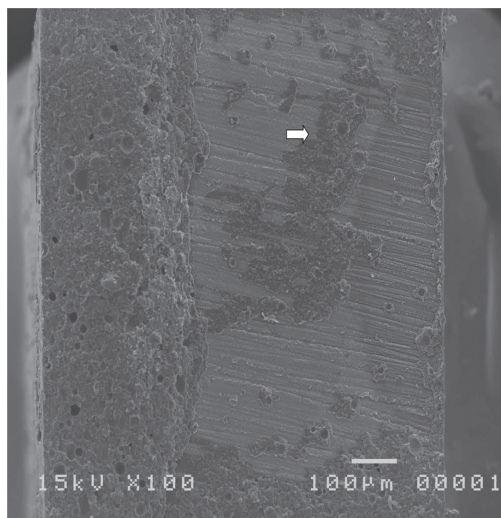


Fig. 3 SEM image showing a mixed failure in SEP/Control specimen along the dentin side, the white arrow points to thin RMGIC area indicating fracture occurring at the base of the restorative material.

LC as well as with using SE primer in this experiment was another reason to select the current positive control groups. Similar future studies using conventional glass ionomers are recommended.

Failure mode analysis in the resin composite groups was predominantly mixed, with higher frequency of adhesive failure in the CR/VIS group. With the RMGIC groups the predominant mode of failure was mixed with CC and SC, and cohesive with SEP with a frequent incidence of dentin cohesive fractures. It was also noticed that with in SEP and SC groups, there was high incidence of fractures at the base of the RMGIC, leaving thin resin surface covering the dentin (white arrow in Fig. 3), suggesting improved RMGIC penetration into dentinal tubules as well as an improvement of the hybrid layer at the RMGIC-dentin interface. It should be noted that bond strength values of all resin composite groups were higher than those of RMGIC groups, which had a higher cohesive and mixed failure in the restorative material. This would seem to indicate that the inherent strength of the material remains an obstacle in achieving bond strength values close to that of resin composite restorations.

CONCLUSION

Based on the results of this study, neither the $AlCl_3$ or $Fe_2(SO_4)_3$ hemostatic agents significantly affected the bond strength of RMGIC to dentin, in addition, using a self-etching primer significantly increased the bond strength of RMGIC compared to either Cavity Conditioner and Self Conditioner.

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