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Application of Calcium Silicate Materials After Acid Etching May Preserve Resin-Dentin Bonds

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Clinical Relevance

Application of calcium silicate materials after acid etching can be a possible solution to preserve the resin-dentin adhesive interface.

SUMMARY

Introduction: The aim of the present study was to evaluate the effect of the application of calcium silicate materials (CSMs), after acid etching, on the longevity of the hybrid layer and marginal adaptation of composite restorations.

Methods and Materials: Eighty human permanent molars received an intrapulpal pressure of 15 cm H_2O. Sixty teeth received a mesial proximal slot preparation with the gingival margin extending 1 mm below the cemento-enamel junction. The samples were divided into two groups. Group 1 received restorations using two types of etch-and-rinse adhesives: ethanol based (Single Bond, 3M ESPE, St Paul, MN, USA) and acetone based (Prime & Bond NT, Dentsply, DeTrey GmbH, Germany). In group 2 samples, a commercially available CSM (ProRoot MTA) was allowed to set before grinding and placing into a distilled water solution. This solution was applied on the cavity floor after acid etching. The surface was washed after 30 seconds followed by application of adhesives and restorations as in group 1. The samples were stored in phosphate-buffered saline for six months, maintaining the intrapulpal pressure. An epoxy replica was made, and the marginal adaptation was evaluated using scanning electron microscopy. The percentage of continuous margin (CM) was recorded for each group. Another 20 samples were used for hybrid layer evaluation. The crowns were ground to expose dentin. Intrapulpal pressure was applied. The samples were divided into two groups and restored similar to samples restored for marginal adaptation evaluation. The samples were longitudinally cut in 1-mm slices. The slices were stored under 15 cm of phosphate-buffered saline to simulate the pulpal pressure. After six months, the adhesive interface was evaluated using a

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scanning electron microscope. Statistical analysis was done with two-way analysis of variance with Holm-Sidak's correction for multiple comparisons.

Results: Application of CSMs improved the marginal adaptation values in both adhesive groups. In group 1, there were areas of incomplete penetration of resins along with evidence of partial degradation of resin tags. Samples receiving CSM application after acid etching demonstrated long and regular resin tags with very few signs of degradation.

Conclusions: Application of CSMs after acid etching can be a potential avenue in preserving the resin-dentin bonds.

INTRODUCTION

Dental resin adhesives help in the bonding of restorations with dental hard tissues. The resin-enamel bonds are strong and durable; however, this is not true with resin-dentin bonds.[1-5] Traditional etch-and-rinse adhesives use acid etching to partially demineralize the dentin surface, exposing the collagen network.[5,6] This is followed by application of primer/adhesive, which penetrates into the spaces created by demineralization and encompasses the exposed collagen network.[6] This active zone, comprising resin adhesive and collagen fibers, is known as the hybrid layer.[3,7] The longevity of the resin-dentin bonds depends on the durability of the hybrid layer. Unfortunately, the hybrid layer is the most vulnerable area of the resin-dentin interface.[3]

Prior to primer/adhesive application, the etched dentin contains 60% to 70% water and approximately 30% collagen network.[3] In an ideal scenario, the entirety of the water content should be replaced by the penetrating resins of the dental adhesives.[3,8] However, the adhesive resins are not able to completely penetrate the full depth of demineralized dentin.[8-14] As a result, an area of uninfiltrated, demineralized dentin remains below the hybrid layer, which is composed of exposed collagen fibers. Hashimoto and others[9] demonstrated that the adhesive resins were not able to penetrate far enough to reach the depth of acid conditioning. This zone corresponded to the deposition of silver nitrate in the hybrid layer in later studies.[10-14] The exposed fibers are prone to the hydrolytic degeneration by activation of endogenous matrix metalloproteinases (MMPs) and cathepsins.[15,16]

To prevent degradation at the hybrid layer, it is desirable that hydrophobic adhesive resins should completely replace the water and penetrate to the full depth of the demineralized dentin. Various methods have been proposed to achieve this goal. Ethanol wet bonding replaced the water in the acid-etched dentin with ethanol.[14] This allowed increased penetration of hydrophobic resins dissolved in ethanol.[14] However, replacing water with ethanol is not easy and was not clinically feasible. Other methods aimed at deactivating the MMPs and cathepsins.[16]

Calcium silicate materials have been introduced in dentistry by Torabinezad and others,[17] who developed mineral trioxide aggregate (MTA). Calcium silicate materials (CSMs) are mainly composed of tricalcium silicates and dicalcium silicates with addition of other minerals.[18] The CSMs set through a hydration reaction producing calcium silicate hydrate (CSH) and calcium hydroxide.[19] The CSMs have shown an ability to remineralize the artificial demineralization.[20] When demineralized dentin slabs were placed in contact with set CSMs, zones of remineralization were reported.[20,21]

The present study hypothesized that the application of CSMs after acid etching may help to prolong the durability of the hybrid layer. The CSH particles may be able to enter the demineralized dentin, replacing and reducing the water content. Also, it may help to deactivate the proteinases by the caustic activity of alkaline pH. Composite restorations were placed with or without application of CSMs after acid etching. Hybrid layers and marginal adaptations were evaluated. The null hypothesis was that application of CSMs did not improve the resin-dentin bonds.

METHODS AND MATERIALS

The study was approved by the Institutional Ethics Committee (proposal 8/4/65/JMI/IEC/2016). The study involved the use of eighty freshly extracted, nonrestored, noncavitated human permanent molars. The teeth had similar crown dimensions. The samples were cleaned with a hand scaler and were stored in distilled water at 4°C until use. All samples were used within one week of their storage. The roots were trimmed perpendicular to the long axis of the tooth until the pulp chamber was exposed. The pulp tissue was gently removed from the chamber. A small hole was prepared in a plexiglass slab ($2 \times 2 \times 0.5$ cm) with a round bur (001/018; Mani Inc, Shioya, Tochigi, Japan). The tooth was glued to the plexiglass slab, in a manner that the hole was placed just over the pulp chamber opening. An 18-gauge needle was placed inside the hole so that it could penetrate

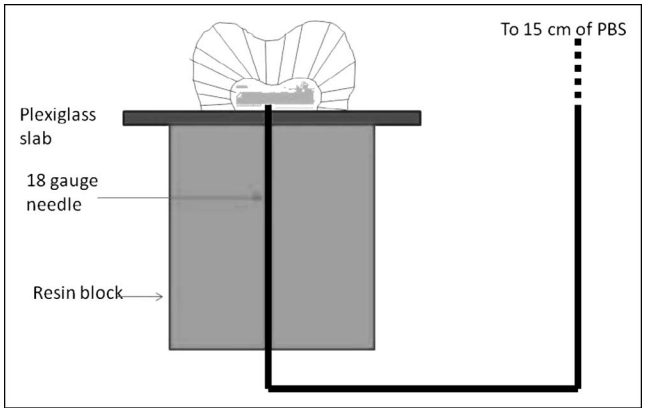

Figure 1. *Setup for simulated intrapulpal pressure. PBS, phosphate-buffered saline.*

into the pulp chamber. The tooth was sealed over the slab with the help of a cynoacrylate glue. The space between the needle and the hole was also sealed. The needle was attached to an intravenous set, which was attached to a bottle of phosphate-buffered saline. The bottle was attached to a pole and elevated 15 cm above the tooth to simulate intrapulpal pressure (Figure 1).

Sixty teeth received a mesial proximal slot preparation. The cavities were prepared using standard (125 μm) and fine (63 μm) diamond burs (straight flat end 111/014, flat end tapered fissure 172/023, and tapered conical end 160/016; Mani Inc, Tochigi, Japan) in a high-speed turbine (KaVo, Germany). The occlusal and gingival dimensions of the isthmus were kept at 3.25 ± 0.25 mm and 4 ± 0.25 mm, respectively. The gingival margin was kept 1 mm below the cementoenamel junction. Each bur was replaced with a new bur after four cavity preparations. The samples were divided into two groups on the basis of CSM application. The groups were further divided into two subgroups on the basis of adhesive system used. The schematic methodology is depicted in Figure 2.

Group 1 (Control Group)

The samples were divided into two subgroups (n=15) on the basis of resin adhesive used. Two types of etch-and-rinse adhesive were used: ethanol based (Single Bond, 3M ESPE, St Paul, MN, USA) and acetone based (Prime & Bond NT, Dentsply, DeTrey GmbH, Germany). The composition and mode of application of the adhesive systems used in this study are presented in Table 1. The light-curing unit, (Bluephase G2, Ivoclar Vivadent, Schaan, Liechtenstein) was operated at an intensity of 1,200 mW/cm^2. The power intensity was regularly checked using a dental radiometer (LM-1 Light Meter, Woodpecker, Guilin, China). After application of adhesive agent, a 1-mm layer of flowable composite (Filtek Z350 XT for the Single Bond group and Esthet-X Flow for the Prime & Bond NT group) was placed and light cured. The remaining cavity

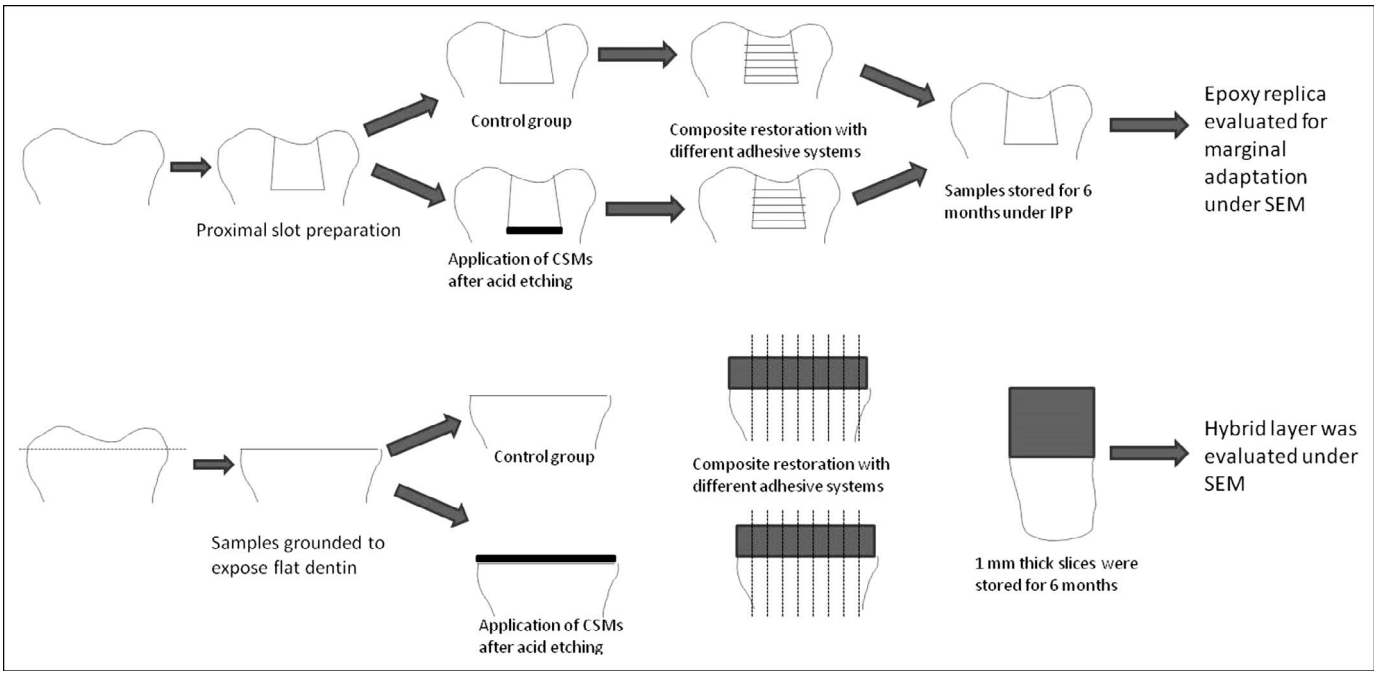

Figure 2. *Schematic methodology. CSM, calcium silicate material; IPP, intrapulpal pressure; SEM, scanning electron microscope.*

Table 1: *Composition and Mode of Application of Materials Used*

	Composition	Mode of Application
Single Bond (3M ESPE, St Paul, MN, USA) Batch No. N831690	Etchant: 35% phosphoric acid gel Adhesive: dimethacrylates, HEMA, polyalkenoid acid copolymer, 5 nm silane-treated colloidal silica, ethanol, water, photoinitiator	1. Apply etchant, wait 15 s, rinse 10 s 2. Blot excess water 3. Apply two coats of adhesive for 15 s 4. Gently air thin for 5 s 5. Light cure for 10 s
Prime & Bond NT Dentsply, DeTrey GmbH, Germany) Batch No. 1612000507	Adhesive: PENTA, TEGDMA, Bis-GMA, cetylamine hydrofluoride, acetone, nanofiller (amorphous silicon dioxide 8 nm), resin R5-62-1, T-resin, D-resin, CQ	1. Apply etchant, wait 15 s, rinse 10 s 2. Blot excess water 3. Apply two coats of adhesive for 15 s 4. Gently air thin for 5 s 5. Light cure for 10 seconds
Filtek Z350 XT flowable resin (3M ESPE, St Paul, MN, USA) Batch No. N33729	BISGMA, TEGDMA, silane-treated ceramic, EDMAB, Ytterbium fluoride	1. After application of bonding agent, 0.5-1 mm of resin applied 2. Light cure for 20 s
Esthet-X Flow (Dentsply, DeTrey GmbH, Germany)	BISGMA Adduct, BIGEMA Adduct, triethylene glycol dimethacrylate, barium floroborosilicate	1. After application of bonding agent, 0.5-1 mm of resin applied 2. Light cure for 20 s
MTA (ProRoot MTA white, Dentsply; Tulsa Dental Specialities, GmbH, Germany) Batch No. 144794	Powder: Dicalcium silicate Tricalcium silicate Bismuth oxide Liquid: Distilled water	1. Powder and liquid mixed with the help of a spatula 2. Material allowed to set for 7 d before use

was restored with a composite restorative material (Z350, 3M ESPE) in 2-mm increments using a clear matrix strip. After curing, the matrix strip was removed and gingival margins contoured with a composite polishing kit (Sof-Lex Finishing and Polishing System, 3M ESPE).

Group 2

The samples were subjected to application of CSMs before application of dental adhesive. A commercially available CSM (ProRoot MTA White, Dentsply; Tulsa Dental Specialities) was used. MTA was mixed according to the manufacturer's recommendations and was allowed to set for seven days at 100% humidity. The set MTA was crushed, and a fine powder was obtained. The cavity surface was etched with 37% phosphoric acid. The acid was washed after 15 seconds, and the cavity surface was kept moist. The set powdered MTA was mixed with distilled water, and a slurry was obtained. The slurry was copiously applied to the cavity surface and was gently rubbed for 30 seconds. The cavity surface was washed with distilled water for 30 seconds. Adhesive systems were applied, and cavities were restored as in group 1.

The samples were stored in phosphate-buffered saline for six months. The solution was changed every month. The intrapulpal pressure was maintained for the whole duration. For evaluation of marginal adaptation, the resin-dentin interface was first cleaned with 37% phosphoric acid for five seconds. Impression of the teeth was made using polyvinyl siloxane impression material (Honigum, DMG, Hamburg, Germany), and replicas were made using epoxy resin (Technovit Epox, Kulzer, Wehrheim, Germany). The replicas were mounted on aluminum stubs and were sputter coated. The gingival margins were analyzed at 65-1500× magnification in a scanning electron microscope (Zeiss, Oberkochen, Germany). The marginal adaptation was classified as "continuous margin" if the interface between the restoration and tooth was continuous and exhibited less than a 1-μm gap and as "gapped margin" if the interface had gaps more than 1-μm wide. The percentage of continuous margin (CM) was recorded for each group. Statistical analysis was performed using two-way analysis of variance (ANOVA) tests keeping the use of CSMs and type of adhesive systems as variables. The level of confidence was kept at 95%.

Evaluation of Hybrid Layer

To evaluate the resin-dentin interface, 20 teeth (n=5, four groups) were evaluated. Before application of intrapulpal pressure, the crowns of the teeth were ground to expose the dentin. The dentin surface

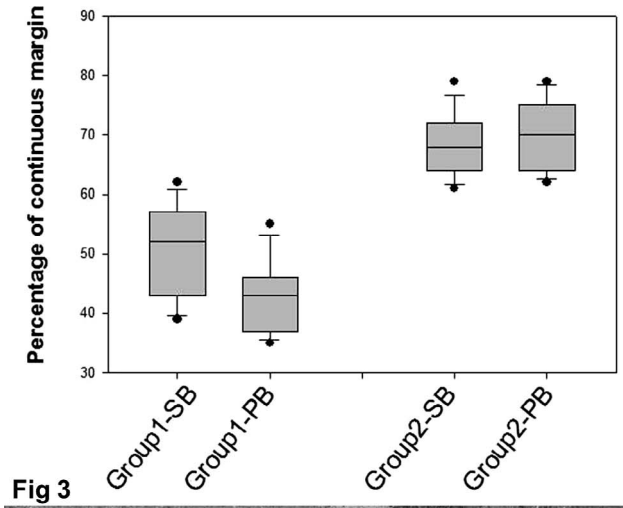

Fig 3

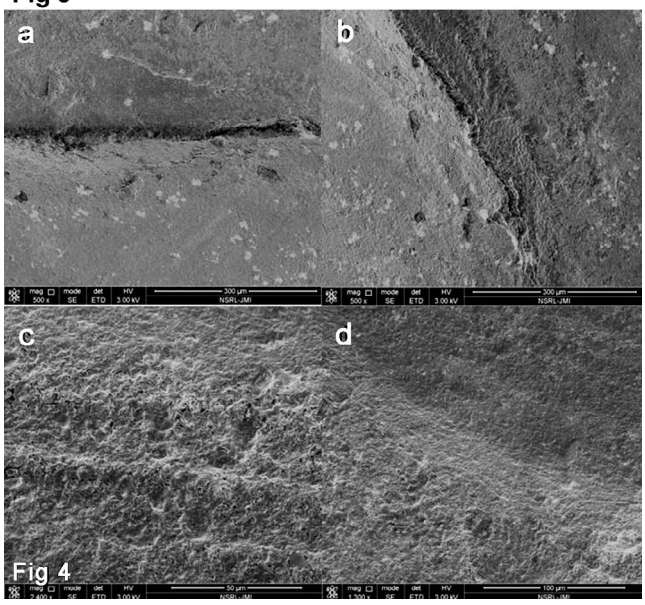

Figure 3. *Box plot graph of values of continuous margins in all groups.*

Figure 4. *Marginal adaptation in epoxy replicas at 500-2400× magnification. (a, b): Gapped margin. (c, d): Continuous margin.*

was ground on wet 600-grit SiC paper for 60 seconds. The samples were divided into two groups: the samples in the first group received acid etching, adhesive application, and restoration similar to the samples restored for marginal adaptation. A 2-mm composite buildup was done. In the second group, set MTA slurry was rubbed and washed after acid etching. The samples were restored using resin composites. The intrapulpal pressure assembly was removed, and the samples were cut into 1-mm slices to expose the resin-dentin interface. The slices were stored under 15 cm of phosphate-buffered saline to simulate the pulpal pressure. After six months, the adhesive interface was evaluated. The slices were immersed in 6N HCL for 30 seconds to demineralize the dentin, followed by rinsing with water for five minutes. Subsequently, the slices were immersed in 3% NaOCl for 10 minutes and were again rinsed with water for five minutes. The specimens were dehydrated and were mounted on aluminum stubs for sputter coating and scanning electron microscope analysis.

RESULTS

There was a significant difference between the values of percentages of CM obtained in different groups; hence, the null hypothesis was rejected ($p<0.05$, two-way ANOVA with Holm-Sidak's correction for multiple comparisons; Table 2). The overall marginal adaptation values in group 1 were 46.6% (Figure 3). There was a significant difference between the CM values of Single Bond (50.25±7.7%) and Prime & Bond NT (43%±6%) subgroups ($p=0.002$). Figures 4a,b show the marginal adaptation in the subgroups of group 1. A gross deterioration of the resin-dentin interface was noticed in most slices. Gaps as wide as 25 μm were noticed. Application of CSMs after acid etching significantly improved the CM values. Overall, the CM values were 69.2%, with no difference between the two subgroups (68.4%±5.2% and 70%±5.5% for Single Bond and Prime & Bond NT, respectively). Figure 4c,d shows some CMs in group 2. There was significantly less marginal deterioration as compared with group 1.

Five samples from each group were evaluated for resin tags and hybrid layer quality. A consistent hybrid layer was noticed in all groups. The thickness of the hybrid layer was 4-7 μm. In group 1, two types of defects were noticed: 1) areas of incomplete penetration of resin denoted by irregular diameter and length of the resin tags (Figure 4a,b) and 2) partial degradation of resin tags suggesting hydro-

Table 2: *Two-Way ANOVA*

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	*F*	*p*
Application of CSM	1	7638	7638	199	<0.001
Different adhesive systems	1	120	120	3.1	0.082
Variable 1 vs variable 2	1	286	286	7.4	0.008

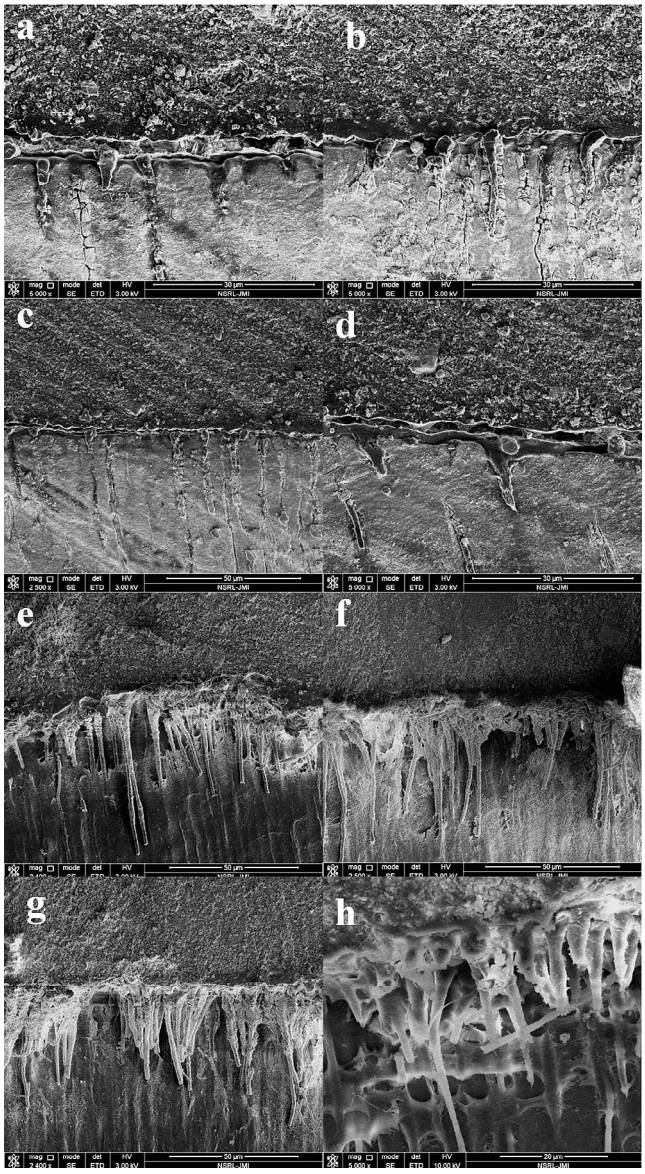

Figure 5. *Hybrid layer evaluation at 2500-5000× magnification. (a-d): Group 1 showing areas of incomplete resin penetration and partial degradation of resin tags. (a, c): Adhesive bond failure. (e-h) Long/ regular resin tags, with minimal sign of degradation.*

lytic degradation of resins (Figure 5a-d). Bond failure was seen at many places, with most of them being adhesive in nature (failure at the junction of the hybrid layer and dentin). In some groups, partial degradation of the hybrid layer, near the dentinal junction, was seen. Samples receiving CSM application after acid etching demonstrated long and regular resin tags with length ranging to tens of microns. The tags were initially funnel shaped and then subsequently tapered until they took a long, cylindrical form. The length and the diameter of the resin tags were consistent with very few signs of degradation (Figure 5e-g). Lateral branching of tags was noticed in some specimens (Figure 5h).

In group 2 specimens, deposition of particles was seen inside the dentinal tubules. An energy dispersive X-ray analysis (EDAX) was performed to ascertain their chemical composition. The analysis showed the presence of calcium, silicate, aluminum, and traces of chloride, phosphorous, and carbon (Figure 6).

DISCUSSION

To simulate intrapulpal pressure, the specimens received 15 cm of water pressure. The dentin is primarily a hydrated structure with dentinal fluid present in the dentinal tubules.[22,23] In a clinical scenario, the blood pressure inside the pulp chamber pushes the dentinal fluid outward toward the cavity floor. This impedes the penetration of resin into the dentinal tubules.[24] It is recommended to simulate the intrapulpal pressure in studies evaluating the resin-dentin interface. Application of simulated intrapulpal pressure has shown to reduce the length of resin tags and negatively affect the tensile bond strength.[24,25] Various authors have used pressures ranging from 5 cm to 36 cm of water.[25-27] Ciucchi and others[23] estimated the pulpal tissue pressure and reported a positive pulpal pressure of 14.1 cm of water in healthy teeth. An infiltration anesthesia with solutions containing epinephrine reduced the pulpal blood flow by 72%.[28] Purk and others[25] reported that application of different amounts of pulpal pressure (5 cm H_2O and 15 cm H_2O) reduced the microtensile bond strength compared with specimens restored without pulpal pressure. However, increasing the pulpal pressure from 5 cm to 15 cm had no effect on the bond strengths.[25]

Two-step etch-and-rinse adhesive systems were used in the present study. The systems differed on the basis of solvents. Prime & Bond NT was acetone based, while Single Bond used ethanol and water as solvents.[29] Acetone has a high vapor pressure and water-removing capacity, often termed a *water-chasing* ability.[29,30] However, while using acetone-based adhesives, care must be taken to evaporate the excess water before light curing the adhesive.[29] The ethanol-based systems usually contain water as a cosolvent. Combined with water, the ethanol solution is azeotropic in nature.[29] In comparing acetone- vs ethanol-based adhesive systems, the literature provides different results. Tay and others[31] observed a "complex phase separation pattern" in specimens restored using an acetone-based adhesive, in the presence of surface moisture. Transmission electron

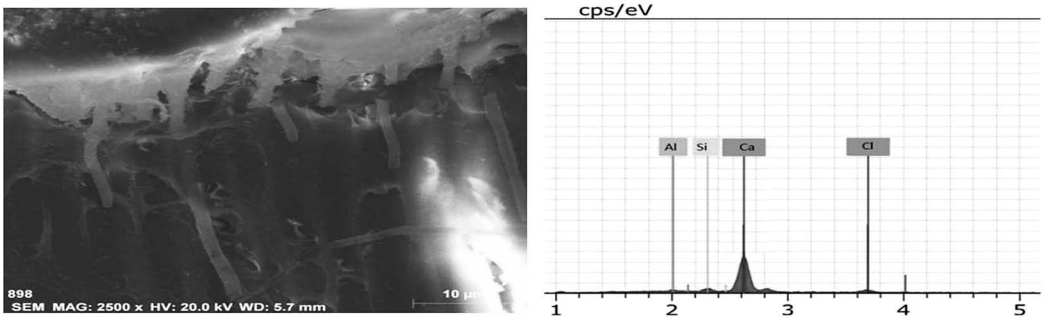

Figure 6. *EDAX analysis of particles deposited inside the dentinal tubules in group 2. The analysis showed presence of calcium, silicate, aluminum, and traces of chloride, phosphorous, and carbon.*

microscope examination revealed that in the presence of excess moisture, the intratubular resin infiltration was compromised.[31] The ethanol-based adhesive performed better in some *in vitro* studies.[24,25] The microtensile bond strength was higher than acetone-based adhesive in the presence of intrapulpal pressure. However, some studies have observed that a pressure of 15 cm H_2O does not compromise the bond strength of acetone-based adhesives.[32] The water-chasing ability was thought to be a reason for the better resin penetration despite perfusion through dentin. In the present study, the Single Bond (ethanol-based) adhesive presented with better marginal adaptation than the Prime & Bond NT (acetone-based) adhesive. The marginal adaptation was evaluated using an epoxy replica of the specimen. This method gives a reliable quantitative assessment of the marginal quality, in terms of continuous or gapped margins.[33,34] Since the specimens have to undergo a vacuum process during scanning electron microscopy, the use of epoxy replica rules out the possibility of formation of cracks, which can be formed on actual teeth if placed in a vacuum chamber.[34]

In both of the adhesive systems, there was a degradation of the hybrid layer. Hashimoto and others[35] observed two patterns of hybrid layer degradation: disorganization of collagen fibers and hydrolysis of resins. In the present study, hybrid layer and resin tags were evaluated after storage of slices for six months. In the present study, there were areas with incomplete penetration of resin into the dentinal tubules along with evidence of degradation of resin tags. This degradation was seen in both adhesive systems. Since the bonded interface was exposed by sequential rinses in hydrochloric acid and sodium hypochlorite, most collagen was removed and resin tags were exposed. Several mechanisms have been proposed to explain the degradation of the hybrid layer. During acid etching, the subsurface mineral is solubilized and extracted.[3] This part gets replaced with water, raising the water content of etched dentin to 70%.[3] In an ideal scenario, this water is replaced by the resin monomers. However, the resins are not able to fully replace the water. This condition gets worse by application of intrapulpal pressure that forces the fluid into dentinal tubules. This leads to presence of local water-rich regions within the hybrid layer, which can be identified as nanoleakage. The presence of water can cause hydrolysis of resins in the hybrid layer.[36,37] The second mechanism of the degradation of the hybrid layer relates to the activation of MMPs.[15] The MMPs are bound to the healthy dentin. During acid etching, the MMPs are released and activated.[15] These enzymes break the collagen fibers by virtue of their collagenase and gelatinase activity. As explained before, the resin does not penetrate fully to the depth of demineralization, exposing some collagen fibers surrounded by water.[8] These exposed fibers are attacked by the MMPs. Another mechanism relates to the presence of proteolglycan hydrogels, which interfere with the infiltration of resins.[16]

It is quite clear that any mechanism that protects the collagen fibers and restricts the movement of fluid inside the hybrid layer will make the adhesive interface more durable.[3] It was hypothesized that application of calcium silicate materials, after acid etching, will help to preserve the resin-dentin interface. The CSMs have many applications in dentistry because of their bioactive properties.[19] The first commercially available CSM was MTA, which consists of calcium silicates and aluminates along with bismuth oxide as a radiopacifier.[18] When set calcium silicate material was exposed to simulated body fluid for seven days, a layer of hydroxyapatite was formed over the CSM.[38] Tay and others[39] reported that when set Portland cement was immersed in phosphate-buffered saline, an

amorphous calcium phosphate (ACP) phase was formed that transformed into an apatite phase. These ACP precursors are subnanometer sized and have a potential to act as prenucleation clusters for the development of oriented apatite crystals.[40]

A commercially available CSM (ProRoot MTA) was used in the present study. The material was allowed to set for seven days and then finely ground. The set material was gently rubbed over the acid-etched dentinal surface. A pilot study revealed that using unset MTA over the dentin surface prevents resin infiltration since it tends to set within minutes of coming in contact with water. The marginal adaptation was better than the samples restored without CSM application. The hybrid layer evaluation revealed that the resin tags were long, parallel in shape, and had minimal signs of degradation. The hybrid layer was intact in most places. There was evidence of deposition of particles inside the dentinal tubules. EDAX analysis revealed that the particles had a chemical composition of calcium, aluminium, and silicate with traces of chloride, phosphorous, and carbon. Gandolfi and others[41] evaluated the effect of application of CSMs on the dentinal discs treated with EDTA and reported that the dentinal tubules were covered with particles having a chemical composition of calcium and phosphate and traces of aluminum, silicate, chloride, and magnesium.

As explained before, a major cause of degradation of the adhesive interface is the presence and flow of water through the dentinal tubules. Application of CSMs have been shown to reduce the permeability of dentin.[41] They also help to provide a precursor for apatite formation that helps to remineralize the dentin. This may also preserve the collagen fibers. The hydration of CSMs yield two main components: calcium-silicate-hydrate and calcium hydroxide.[18,19] When the set CSMs are in contact with phosphate-containing solutions, the calcium-silicate-hydrate leads to precipitation of ACP that transforms into carbonated apatite.[39] The calcium hydroxide has an alkaline pH, which can denature the enzymes.[42] It is possible that application of CSMs could have reduced the activity of MMPs by virtue of their alkaline pH.

There are a few limitations to the present study. The resin-dentin margins were not scanned before the aging process. This could have helped us in understanding the ill effects of aging on the resin-dentin interface. However, this phenomenon has been reported extensively in the literature. The aim of the study was to develop a strategy to negate this ill effect. Some researchers have raised doubts about the validity of scanning electron microscopy analysis. However, scanning electron microscopy has been a backbone of the research conducted on the analysis of resin-dentin interface.[1,2,6,7,8,10,14-16,27,28]

CONCLUSIONS

Application of CSMs after acid etching can be a possible solution to preserve the rein-dentin adhesive interface. Further studies, involving microtensile bond strength evaluation and future clinical trials, are required to evaluate the long-term effect of CSMs on demineralized dentin, and if promising results are obtained, a commercial formulation can be developed.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of approval of the Jamia Millia Islamia Institutional Ethics Committee. The approval code for this study is 8/4/65/JMI/IEC/2016.

Conflict of Interest

The authors of this article certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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