Influence of Adhesive Application Methods and Rebonding Agent Application on Sealing Effectiveness of All-in-One Self-Etching Adhesives

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ABSTRACT

Statement of Problem: The choice of adhesive application methods could affect the microleakage of self-etch adhesives.
Purpose: To evaluate the effect of acid-etching, doubling adhesive application time, doubling adhesive coating, and rebonding agent application on microleakage of self-etch adhesives in Class V cavities.
Materials and Methods: Seventy human third molars with Class V cavities assigned to five groups according to different adhesive application protocols for the three dentin adhesives (Clearfil S3 Bond, Kuraray Medical, Okayama, Japan; Optibond All-in-One, Kerr Corporation Orange, CA, USA; G-Aenial Bond, GC Corporation, Tokyo, Japan): group 1, manufacturer's recommendations; group 2, prior acid-etching of cavities; group 3, double application time; group 4, two consecutive coats of the adhesives; group 5, rebonding application on restoration margins. After bonding, the cavities were filled with a resin composite (Filtek Supreme XT, 3M ESPE Dental Products, St. Paul, MN, USA). The teeth were thermocycled, and the specimens were examined for microleakage using methylene blue as a marker.
Results: For Clearfil S3 Bond and Optibond All-in-One, microleakage in groups 2 and 5 were significantly lower than other groups' enamel margins. In groups 1, 2, 4, and 5, Clearfil S3 Bond exhibited significantly more leakage than the other dentin bonding agents in dentin margins. Microleakage was significantly higher on dentinal margins compared with the enamel margins for Clearfil S3 Bond in all of the groups. Optibond All-in-One showed significantly lower microleakage in dentin margins in all groups except groups 2 and 5.
Conclusion: Acid-etching usually promoted the reduction of microleakage in enamel margins. On the other hand, rebonding application usually contributed to the reduction of microleakage more than other methods in enamel and dentin margins.

CLINICAL RELEVANCE

Acid-etching or rebonding application may contribute to reduction of microleakage of all-in-one self-etching adhesives.

INTRODUCTION

Since the introduction of the enamel etch technique by Buonocore,[1] adhesive techniques have been developed to such an extent that they are now involved in most of the clinical procedures.[2] The basic mechanism of bonding to enamel and dentin is essentially an exchange process involving the replacement of minerals removed from the hard dental tissue by resin monomers that *in situ* become micromechanically

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interlocking in the created porosites.[3] Besides the number of application steps, adhesives can be classified based on the underlying adhesion strategy in "etch-and-rinse," "self-etch," and "resin-modified glass-ionomer adhesives."[4]

Several simplified, one-bottle and self-etching primer systems have recently been introduced to decrease the technique sensitivity. In the one-bottle system, primer and adhesive are combined in one solution. In self-etching primer systems, unsaturated, potentially polymerizable organic acids, or acidic monomers are incorporated, and etching and priming of the dentin occur simultaneously. They are based on infiltration and modification of the smear layer and demineralization of the underlying outer dentin layer of the dentin, followed by infiltration of the smear layer-covered dentin with acidic monomers of the primer.[5] In one type of self-etching primer system, an adhesive is applied on top; in the "all-in-one" systems, all three steps are applied simultaneously.[4]

The use of acid-etching to modify the enamel structure with phosphoric acid[1] has become a standard procedure for conditioning enamel prior to bonding agent application.[6] The infiltration of adhesive resin into the porous zone results in the formation of tags, thereby establishing micromechanical retention to the etched enamel.[7–9] Single-step, self-etch adhesive systems form a continuous layer by demineralization with acidic monomers, followed by resin monomer penetration into the enamel surface. Penetration of the acidic monomers into etched enamel creates resin tags. It was reported that because of their higher pH, self-etch adhesives result in a shallow enamel demineralization compared with that of phosphoric acid.[10,11] Therefore, a separate phosphoric acid enamel-etching step may enhance the bond strengths of self-etch adhesives.[12–14] Because the pH of the self-etch adhesives is not as acidic as the phosphoric acid used with the etch-and-rinse adhesives,[10] concerns have been raised regarding the performance of self-etch adhesives on enamel.[15] Several in vitro investigations have reported low resin-enamel bond strengths of two-step and all-in-one self-etch adhesives.[10,16–19]

The high quality of the hybridization process depends on optimal monomer infiltration between the collagen fibrils of the demineralized matrix and the removal of as much water and organic solvents as possible from the surface prior to curing.[20,21] One possible technique for optimizing the resin monomer infiltration and subsequently creating a more stable and stronger bond is lengthening its diffusion time.[22] Recent studies demonstrated that the more prolonged the application time of simplified adhesives, the higher the immediate resin-dentin bond strength values.[23–25]

In order to create a relatively thick intermediate layer with low elastic modulus between dentin and composite, with the objective of absorbing shrinkage stresses through an elastic deformation, one option is to apply a second adhesive layer.[26–28] It was reported that one way to improve the demineralization effect and hybrid layer quality was to increase acidic monomer concentration by applying additional coats of adhesive.[29–31] The higher the supply of new monomer molecules and acidic moieties to the applied adhesive layer, the higher the etching potential and monomer infiltration giving both the hybrid and the adhesive layer better cross-linking and mechanical properties.[30,31]

The concept of rebonding to seal marginal gaps consists of applying an unfilled resin bonding agent over the margins of the finished restorations to compensate for the adverse effect of the polymerization shrinkage on the tooth/restoration interface and to guarantee higher quality and durability of the marginal adaptation.[32–35] Penetration of the unfilled resin into interfacial microgaps by capillary action, especially in the dentin and cementum margins, would seal the marginal gaps and reduce the microleakage.[34,36] Rebonding with low-viscosity resins applied to the margins of polymerized Class V composites has been reported as a practical clinical method to seal contraction gaps.[33] Significant reduction in microleakage has been reported with rebonding of enamel margins[32,33] and gingival margins.[37]

No study has so far compared the combined influence of acid-etching, doubling adhesive application time, doubling adhesive coating, and rebonding agent

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application on microleakage. The aim of this study was to evaluate the effect of acid-etching, doubling adhesive application time, doubling adhesive coating, and rebonding agent application on microleakage of three different solvent-based adhesive systems in Class V cavities.

METHODS AND MATERIALS

Seventy sound human third molars extracted for clinical reasons were selected for this study. After extraction, they were hand-scaled to remove tissue remnants and stored in 0.5% aqueous chloramine T solution under refrigeration until use. Class V cavities were prepared at the cemento-enamel junction on the buccal and lingual surfaces of each tooth, half in the enamel and half in the dentin tissues, using a rounded cylinder diamond bur (Jota Ag Rotary Instruments, Ruthi, Switzerland, ISO no. 806,314,140,534,012) at high speed with air/water spray. The cavity preparations were standardized with a width of 4 mm, a depth of 2 mm, and a height of 2 mm. These distances were measured with a digital caliper (Digital Slide Caliper, Tchibo GmbH, Hamburg, Germany). Margins of the cavities were butt-jointed with the occlusal margin in enamel and the gingival margin in dentin. The prepared cavities were randomly assigned to five experimental groups of three tested dentin adhesives (the number of cavities for each group was seven, and the number of cavities for each dentin adhesive was 35, for a total of 105 cavities):

1. Clearfil S3 Bond (Kuraray Medical, Okayama, Japan)
2. Optibond All-in-One (Kerr Corporation, Orange, CA, USA)
3. G-Aenial Bond (GC Corporation, Tokyo, Japan)

The dentin bonding agents were applied on enamel and dentin following five different protocols ($N = 105$ cavities totally). The materials and studied groups are described in Tables 1 and 2, respectively.

Group 1 (Control) (G1): Three dentin bonding agents were applied and photopolymerizated strictly according to manufacturer's recommendations (Table 2).

Group 2 (G2): Cavity was etched with 37% phosphoric acid gel (Prime Dent Etchant Gel 37%, Prime Dental Manufacturing, Inc., Chicago, IL, USA) for 15 seconds, rinsed and gently dried with compressed air for 15 seconds. Then, dentin bonding agents were applied and photopolymerizated in the same manner as group 1.

Group 3 (G3): One coat of dentin bonding agent was applied with double application time that was recommended by manufacturers for each adhesive system before photopolymerization (Table 2). The mode of application for each dentin bonding agent followed the manufacturer's instructions for a double application time.

Group 4 (G4): The three dentin bonding agents were applied in two consecutive coats before photopolymerization. The application procedure of a first coat was performed according to the

TABLE 1. Ingredients, manufacturers of dentin bonding systems used in this study

Dentin bonding agents	Manufacturer	Ingredients
Clearfil S3 Bond	Kuraray Medical, Okayama, Japan	Bisphenol A diglycidylmethacrylate, 2-hydroxyethyl methacrylate, 10-methacryloyloxydecyl dihydrogen phosphate, ethanol, colloidal silica, dl-camphorquinone, water, initiators, accelerators
Optibond All-in-One	Kerr, Orange, CA, USA	Acetone, ethyl alcohol, uncured methacrylate ester monomers, inert mineral fillers, ytterbium fluoride, photoinitiators, accelerators, stabilizers, water
G-Aenial Bond	GC, Tokyo, Japan	Acetone, distilled water, dimethacrylate, 4-methacryloxyethyltrimellitate anhydride, phosphoric acid ester monomer, silicon dioxide, photo initiator

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TABLE 2. Application mode of tested groups of three dentin adhesives

Dentin bonding agents	Group 1: control (manufacturer's recommendations)	Group 2: prior acid-etching of cavity	Group 3: doubling application time	Group 4: two consecutive coats	Group 5: rebonding agent application
Clearfil S3 Bond	1. Dispense into container. 2. Apply adhesive. 3. Leave for 20 seconds. 4. Dry by high pressure blowing for more than 5 seconds. 5. Light-cure for 10 seconds.	1. Cavity etching with 37% phosphoric acid gel for 15 seconds. 2. Steps 1 to 5 from group 1.	1. Steps 1 to 2 from group 1. 2. Leave for 40 seconds. 3. Steps 4 to 5 from group 1.	1. Steps 1 to 3 from group 1. 2. Steps 2 to 5 from group 1.	1. Steps 1 to 5 from group 1. 2. Polishing of restorations. 3. The surface and adjacent margins of restorations etching with 37% phosphoric acid gel for 15 seconds. 4. Apply rebonding agent. 5. Light-cure for 20 seconds.
Optibond All-in-One	1. Shake adhesive bottle. 2. Dispense into a clean well. 3. Apply adhesive. 4. Scrub with a brushing motion for 20 seconds. 5. Apply adhesive second. 6. Scrub for 20 seconds. 7. First, dry with oil-free gentle air and then with medium air for at least 5 seconds. 8. Light-cure for 10 seconds.	1. Cavity etching with 37% phosphoric acid gel for 15 seconds. 2. Steps 1 to 8 from group 1.	1. Steps 1 to 3 from group 1. 2. Scrub with a brushing motion for 40 seconds. 3. Apply adhesive second. 4. Scrub for 40 seconds. 5. Steps 7 to 8 from group 1.	1. Steps 1 to 6 from group 1. 2. Steps 3 to 8 from group 1.	1. Steps 1 to 8 from group 1. 2. Polishing of restorations. 3. The surface and adjacent margins of restorations etching with 37% phosphoric acid gel for 15 seconds. 4. Apply rebonding agent. 5. Light-cure for 10 seconds.
G-Aenial Bond	1. Shake adhesive bottle. 2. Dispense into a clean well. 3. Apply adhesive. 4. Leave for 10 seconds. 5. Dry thoroughly for 5 seconds with oil-free air under maximum air pressure. 6. Light-cure for 10 seconds.	1. Cavity etching with 37% phosphoric acid gel for 15 seconds. 2. Steps 1 to 6 from group 1.	1. Steps 1 to 3 from group 1. 2. Leave for 20 seconds. 3. Steps 5 to 6 from group 1.	1. Steps 1 to 4 from group 1. 2. Steps 3 to 6 from group 1.	1. Steps 1 to 6 from group 1. 2. Polishing of restorations. 3. The surface and adjacent margins of restorations etching with 37% phosphoric acid gel for 15 seconds. 4. Apply rebonding agent. 5. Light-cure for 20 seconds.

manufacturer's instructions (Table 2). Then, a second coat was also applied in the same manner as the first coat.

Group 5 (G5): After adhesive application and photopolymerization in the same manner as in group 1, and polishing of restorations, a rebonding agent was applied. The surface and adjacent margins of restorations (2 mm beyond the tooth/restoration interface) were etched with 37% phosphoric acid gel for 15 seconds, rinsed for 15 seconds, gently dried with compressed air, then the rebonding agent Fortify Plus (BISCO, Inc., Shamburg, IL, USA) was applied and light-cured for 20 seconds according to manufacturer's specifications.

The cavities were filled with nano-filled resin composite Filtek Supreme XT (3M ESPE Dental Products, St. Paul, MN, USA) in one increment and cured for 20 seconds. Polishing of the restorations was performed with Sof-Lex flexible, aluminum oxide discs (3M ESPE Dental Products) of decreasing abrasiveness. The specimens were stored in distilled water at 37°C for 24 hours and then thermocycled 500 times between 5 and 55°C, with a dwell time of 30 seconds. After thermocycling, the apices of all teeth were sealed with amalgam (YDA Amalgam Alloy Capsules, Hangzhou Yinya New Materials Co. Ltd, Hangzhou, China), and two coats of nail polish were applied to within approximately 1 mm of the tooth/composite interface. Then, the specimens were immersed in a 0.5% aqueous solution of methylene blue at room temperature for 24 hours. After removal from the dye, any surface-adhered dye was carefully rinsed away using tap water.

On each restoration, three buccolingual cuts (mesial, middle, and distal) were prepared longitudinally in a buccolingual direction with a diamond saw mounted in a cutting machine (Isomet, Buehler, Lake Bluff, IL, USA) (Figure 1). These preparations yielded six evaluating surfaces (four sections) for each restoration for a total of 630 viewing surfaces. Each specimen allowed one measure in enamel and one in dentin, for a total 1,260 measures, 420 for each dentin adhesive and 84 for each group of each of dentin adhesive. The sections were observed under a stereomicroscope (Olympus SZ61, Olympus Corporation, Tokyo, Japan) at 40× magnification and microleakage at the occlusal

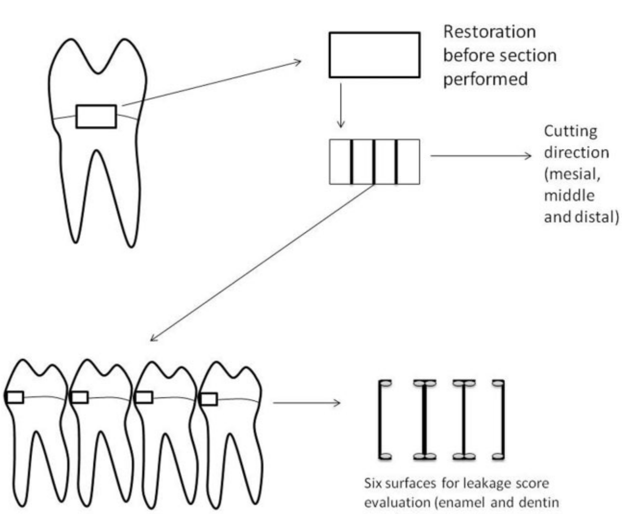

FIGURE 1. Schematic illustration of cutting restorations on the teeth and methodology of microleakage evaluation.

and gingival walls in each section was evaluated by two independent operators according to the following scoring system:

0 = No dye penetration
1 = Dye penetration up to dentinoenamel junction (DEJ) in occlusal margin, or up to one-third of the full length of cervical margin wall
2 = Dye penetration beyond DEJ and up to two-thirds of the full length of the occlusal margin wall, or between one- and two-thirds of the full length of cervical margin
3 = Dye penetration beyond two-thirds of the full length of the occlusal or cervical wall but not involving the axial wall
4 = Extensive dye penetration extending to the axial wall

For each dentin bonding agent, one tooth of each group ($N = 15$) was used for scanning electron microscope (SEM) observation of the resin-dentin interface. Preparation for SEM was as follows: the specimens were sectioned vertically in a buccolingual plane through the center of the restoration and were polished with 600-, 800-, and 1,200-grid silicon carbide abrasive papers under running water, and then for high polishing, they were treated with 1-, 0.3-, and 0.05μm alumina powder slurry using polishing cloths. The specimens were immersed into 10% phosphoric acid solution for 15 seconds, and then they were rinsed with water for 15 seconds and dried for 10 seconds. Afterwards, specimens were treated with 10% sodium hypochlorite for 30 seconds, rinsed thoroughly with water and fixed in glutaraldehyde solution (pH 7.4) for 2 hours. Then, they were dehydrated through ascending series of ethanol (25–100%) and dried at room temperature for 24 hours. Following the drying procedure, the samples were sputter-coated with gold (Emitech K-550X sputter coater, Emitech, Ashford, UK), operating at 20 kV under SEM (JEOL JCM-5000 NeoScope™, JEOL, Tokyo, Japan) with various magnifications.

Statistical analysis was carried out using the Kruskal–Wallis test to determine statistically significant differences in leakage at the occlusal and gingival margins separately among groups for each dentin

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adhesive and between the three dentin adhesives for the same groups. If a significant difference was observed at any margin location, a Dunn multiple comparison test was performed. An intergroup comparison of occlusal versus gingival margin locations was completed using the Wilcoxon signed-rank test. All the statistical tests were performed at a $P < 0.05$ level of significance.

RESULTS

Data of distribution, the mean values, and standard deviations of enamel and dentin microleakage for each group of studied dentin bonding agents and pairwise comparisons are shown in Tables 3, 4, and 5.

On the enamel margins, when comparing groups within each material (Table 3), groups 2 and 5 showed significantly less microleakage than groups 1, 3, and 4 for Clearfil S3 Bond. Also, there was a statistically significant difference between group 1, and groups 4 and 5. For Optibond All-in-One, groups 2 and 5 exhibited significantly less microleakage than groups 1, 3, and 4 (Table 4). Furthermore, groups 1, 3, and 4 demonstrated significantly more leakage than group 2 for G-Aenial Bond (Table 5).

TABLE 3. Distribution of microleakage scores, the mean values and standard deviations of enamel and dentin microleakage for Clearfil S3 Bond dentin bonding agent, and pairwise comparisons

Dentin bonding agent	Groups	N	Enamel leakage scores							Dentin leakage scores						
			0	1	2	3	4	Mean (standard deviation)	Dunn test	0	1	2	3	4	Mean (standard deviation)	Dunn test
Clearfil S3 Bond	G1	42	15	23	4	–	–	0.74 (0.627)	Aa	–	2	6	14	20	3.24 (0.878)	Ab
	G2	42	31	10	1	–	–	0.29 (0.508)	Ba	–	1	2	17	21	3.40 (0.700)	Ab
	G3	42	4	27	11	–	–	1.17 (0.580)	Ca	14	5	2	2	19	2.17 (1.833)	Bb
	G4	42	1	35	6	–	–	1.12 (0.395)	Ca	14	4	–	1	23	2.36 (1.898)	Bb
	G5	42	32	10	–	–	–	0.24 (0.431)	Ba	11	4	1	13	13	2.31 (1.630)	Bb

Within a column, values having different capital letters exhibited statistically significant differences ($P < 0.05$). Within a row, values having different lowercase letters exhibited statistically significant difference ($P < 0.05$), comparison of the same groups between enamel and dentin.

TABLE 4. Distribution of microleakage scores, the mean values and standard deviations of enamel and dentin microleakage for Optibond All-in-One dentin bonding agent, and pairwise comparisons

Dentin bonding agent	Groups	N	Enamel leakage scores							Dentin leakage scores						
			0	1	2	3	4	Mean (standard deviation)	Dunn test	0	1	2	3	4	Mean (standard deviation)	Dunn test
Optibond All-in-One	G1	42	7	19	16	–	–	1.21 (0.717)	Aa	28	12	2	–	–	0.38 (0.582)	Ab
	G2	42	18	23	1	–	–	0.60 (0.544)	Ba	24	16	–	–	2	0.57 (0.914)	Aa
	G3	42	1	35	6	–	–	1.12 (0.395)	Aa	25	17	–	–	–	0.40 (0.497)	Ab
	G4	42	–	37	5	–	–	1.12 (0.328)	Aa	28	14	–	–	–	0.33 (0.477)	Ab
	G5	42	18	21	3	–	–	0.64 (0.618)	Ba	28	12	–	–	2	0.48 (0.917)	Aa

Within a column, values having different capital letters exhibited statistically significant differences ($P < 0.05$). Within a row, values having different lowercase letters exhibited statistically significant difference ($P < 0.05$), comparison of the same groups between enamel and dentin.

TABLE 5. Distribution of microleakage scores, the mean values and standard deviations of enamel and dentin microleakage for G-Aenial Bond dentin bonding agent, and pairwise comparisons

Dentin bonding agent	Groups	*N*	Enamel leakage scores							Dentin leakage scores						
			0	1	2	3	4	Mean (standard deviation)	Dunn test	0	1	2	3	4	Mean (standard deviation)	Dunn test
G-Aenial Bond	G1	42	6	33	3	–	–	0.93 (0.463)	ACa	30	7	–	–	5	0.64 (1.303)	Aa
	G2	42	15	27	–	–	–	0.64 (0.485)	Ba	32	9	–	1	–	0.26 (0.497)	Ab
	G3	42	4	29	9	–	–	1.12 (0.550)	Aa	10	23	4	4	1	1.12 (0.968)	Ba
	G4	42	7	19	16	–	–	1.21 (0.717)	ACa	17	16	1	6	2	1.05 (1.209)	Ba
	G5	42	12	22	8	–	–	0.90 (0.692)	BCa	26	16	–	–	–	0.38 (0.492)	Ab

Within a column, values having different capital letters exhibited statistically significant differences ($P < 0.05$). Within a row, values having different lowercase letters exhibited statistically significant difference ($P < 0.05$), comparison of the same groups between enamel and dentin.

In dentin margins (Table 3), groups 1 and 2 exhibited significantly more leakage than groups 3, 4, and 5 for Clearfil S3 Bond. For Optibond All-in-One, there were not statistically significant differences between all of the groups (Table 4). Groups 2 and 5 showed significantly less leakage than groups 3 and 4 for G-Aenial Bond (Table 5).

For Clearfil S3 Bond, statistically significant differences were determined between enamel and dentin microleakage scores in all of the groups. For Optibond All-in-One, there were also statistically significant differences between enamel and dentin microleakage values for all groups except groups 2 and 5. On the other hand, significant differences between enamel and dentin microleakage were observed in groups 2 and 5 for G-Aenial Bond.

For group 1 (Table 6), there was a significant difference between Clearfil S3 Bond and Optibond All-in-One in enamel margins. Also, Clearfil S3 Bond showed significantly less leakage than other dentin bonding agents in enamel margins for groups 2 and 5. On the other hand, there was not a statistically significant difference between Optibond All-in-One and G-Aenial Bond in enamel margins for all groups. When comparing the three dentin bonding agents for all groups, Clearfil S3 Bond exhibited significantly more leakage than the other dentin bonding agents in dentinal margins except group 3. However, Optibond All-in-One showed significantly less microleakage than the other dentin bonding agents in dentin margins for groups 3 and 4. Clearfil S3 Bond exhibited significantly more leakage than Optibond All-in-One in dentin margins for group 4. Furthermore, there was not a statistically significant difference between Optibond All-in-One and G-Aenial Bond in dentin margins for group 5.

TABLE 6. Comparison of the same groups between three dentin bonding agents

	Dentin bonding agents	G1	G2	G3	G4	G5
Enamel	Clearfil S3 Bond	A	A	A	A	A
	Optibond All-in-One	B	B	A	A	B
	G-Aenial Bond	AB	B	A	A	B
Dentin	Clearfil S3 Bond	A	A	A	A	A
	Optibond All-in-One	B	B	B	B	B
	G-Aenial Bond	B	B	A	C	B

Within a column, values having different capital letters exhibited statistically significant difference for enamel and dentin margins, separately ($P < 0.05$), comparison of the same groups between three dentin bonding agents.

SEM images of the interfaces treated with Clearfil S3 Bond, Optibond All-in-One and G-Aenial Bond for five groups are shown in Figures 2 to 16. For Clearfil S3 Bond, there are gaps in some areas in the enamel and

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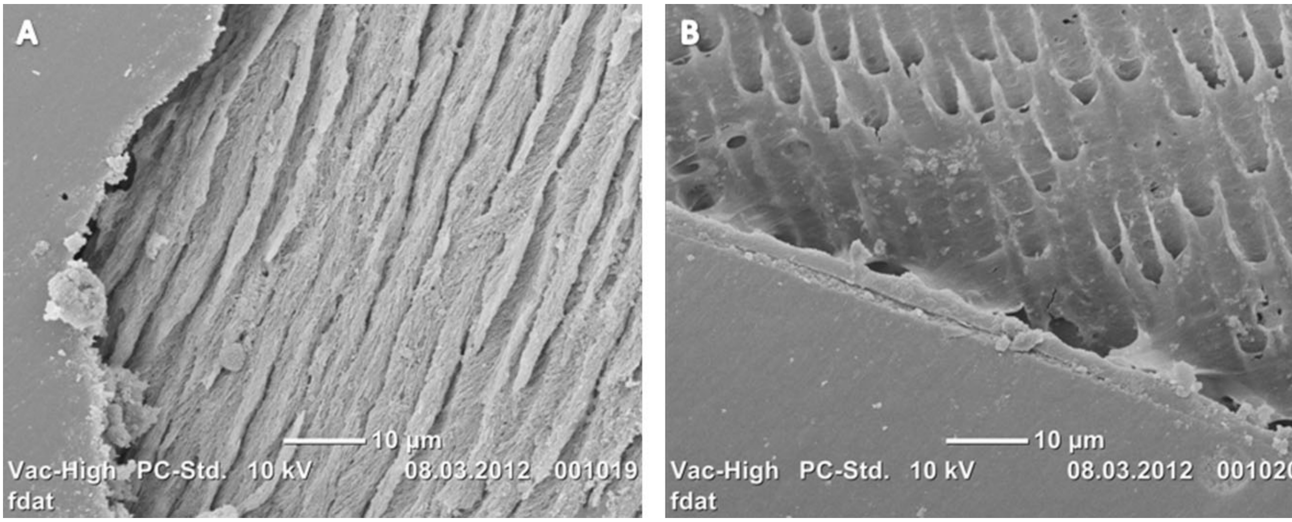

FIGURE 2. Scanning electron microscope image of A, enamel–Clearfil S3 Bond and B, dentin–Clearfil S3 Bond interface of control groups.

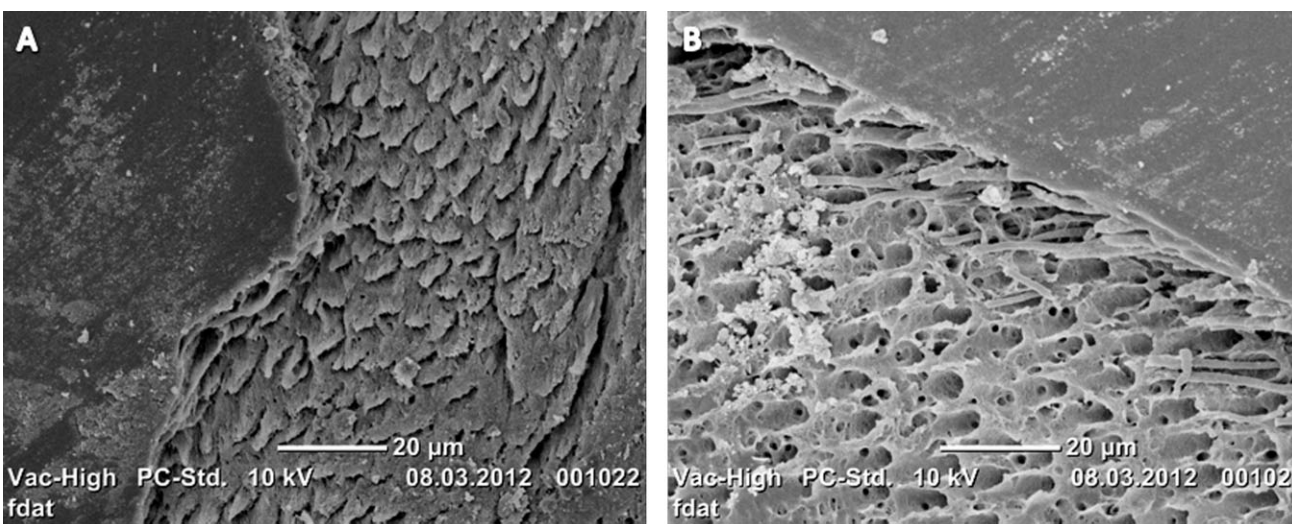

FIGURE 3. Scanning electron microscope image of A, enamel–Clearfil S3 Bond and B, dentin–Clearfil S3 Bond interface of prior acid-etching application groups.

dentin (Figures 2A and B) applied according to manufacturer's instructions (Control Group).
Figures 3A and B show good interfacial adaptation with adhesive layer and resin tags in enamel and dentin after acid-etching application. Generalized gaps can be observed with no resin tags after doubling the application time or using two consecutive coat applications of Clearfil S3 Bond (Figures 4 and 5). Interface shows no gaps and is occluded by rebonding agent in enamel (Figure 6A). Gaps can be observed with a few as well as long resin tags in the dentin (Figure 6B). For Optibond All-in-One, there was good interfacial adaptation with the adhesive layer and scarce resin tags in the enamel and dentin of the control groups (Figures 7A and B). Figures 8A and B show tight interfacial adaptation in the enamel and dentin with pronounced adhesive layer and dense resin tags after acid-etching application. No gap can be observed with resin tags and adhesive layer after doubling the application time or two consecutive coat applications

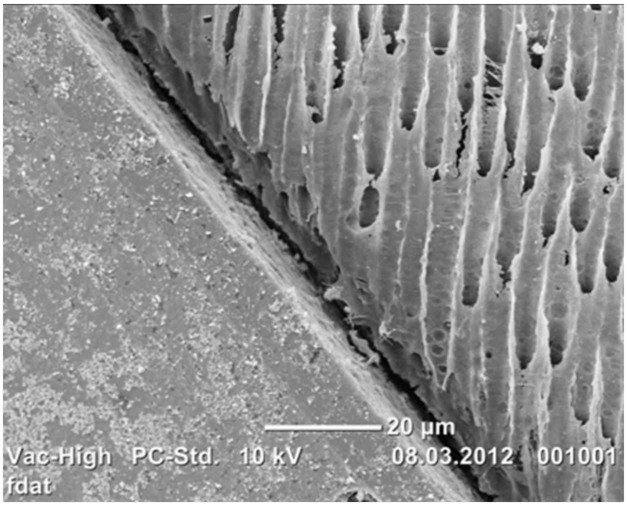

FIGURE 4. Scanning electron microscope image of dentin–Clearfil S3 Bond interface of doubling application time group.

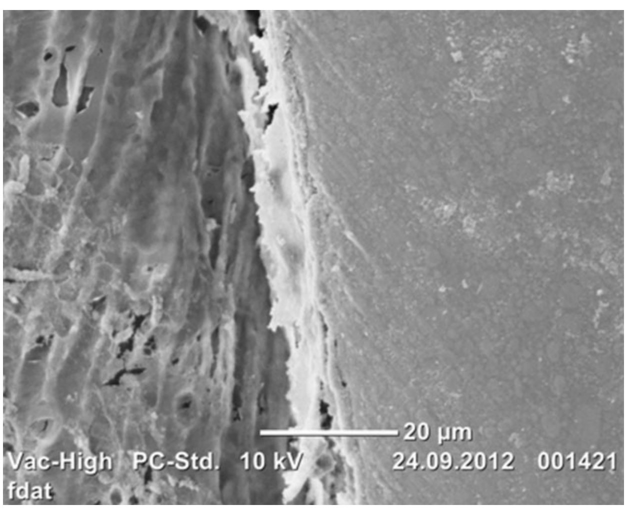

FIGURE 5. Scanning electron microscope image of dentin–Clearfil S3 Bond interface of two consecutive coats groups.

with scarce resin tags for Optibond All-in-One (Figures 9 and 10). Interface shows no gap and is occluded by rebonding agent in enamel (Figure 11A). Also, no gap can be observed with a few as well as long resin tags in the dentin after rebonding agent application (Figure 11B). For G-Aenial Bond, good interfacial adaptation is seen in dentin with adhesive layer and resin tags applied according to manufacturer's instructions (Control Group) (Figures 12A and B) and after acid-etching application (Figures 13A and B). There are gaps in some areas in the dentin after doubling the application time and after two consecutive coat applications with scarce resin tags and adhesive layer (Figures 14 and 15). Interface shows no gap and is occluded by rebonding agent in surface enamel. However, gaps in some areas of deeper enamel are seen (Figure 16A). Also, Figure 16B shows gaps in some dentinal areas with an increase in the number of short resin tags.

DISCUSSION

Even though new materials have been introduced onto the market, the problem of adequate composite resin marginal adaptation and microleakage elimination is far from being solved. The enamel-dentin adhesive systems and their application techniques might play an important role in this area.[38]

Microleakage was significantly higher on dentinal margins compared with the enamel margins for Clearfil S3 Bond in all of the groups. On the other hand, microleakage was significantly higher on enamel margins compared with dentin margins for Optibond All-in-One in all of the groups, except the groups that employed etching of enamel and dentin, or rebonding of enamel and dentin margins. But when the three dentin adhesives were applied according to the manufacturer's recommendations, Clearfil S3 Bond showed significantly more microlekage than other adhesives on dentinal margins. In contrast with the present study results, Owens and Johnson observed that Clearfil S3 Bond showed slightly less leakage at the apical margins (mean score 1.042) than coronal margins (mean score 1.542) in Class V cavities.[39] The Clearfil S3 Bond contains 10-metacryloxydecyl dihydrogen phosphate (MDP), which has been reported to have a high chemical bonding potential to hydroxyapatite.[40] Furthermore, the calcium salt of MDP is highly insoluble.[40] According to the adhesion-decalcification concept, the less soluble the calcium salt of an acidic molecule, the more intense and stable the molecular adhesion to a hydroxyapatite substrate.[41] Because of the high mineral content of enamel, MDP may interact with enamel more intensely, and this may contribute to less leakage on the enamel margins than dentinal

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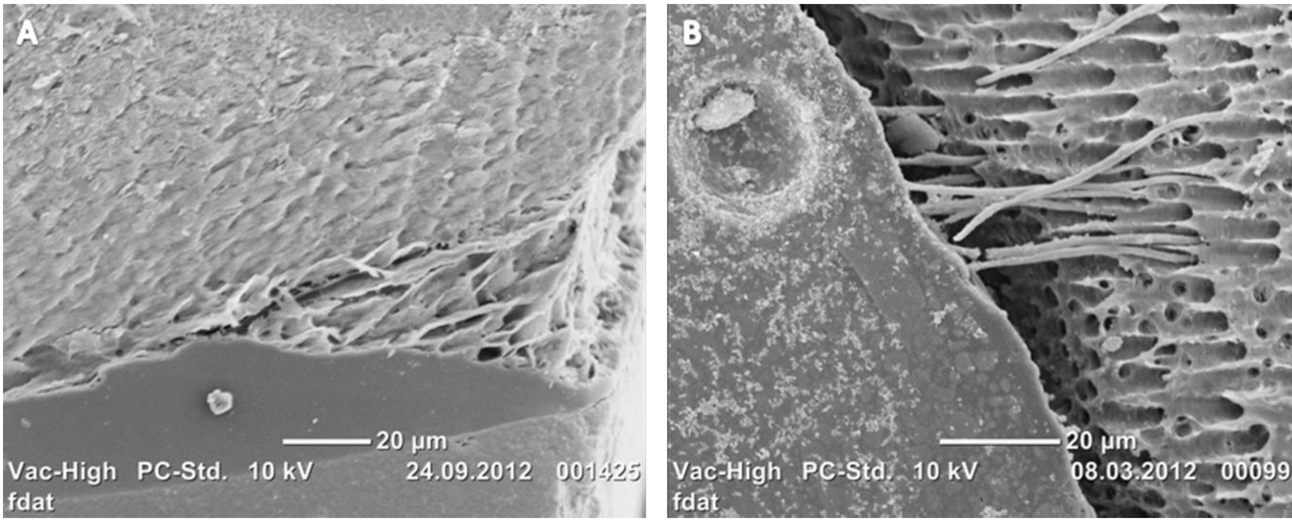

FIGURE 6. Scanning electron microscope image of A, enamel–Clearfil S3 Bond and B, dentin–Clearfil S3 Bond interface of rebonding agent application groups.

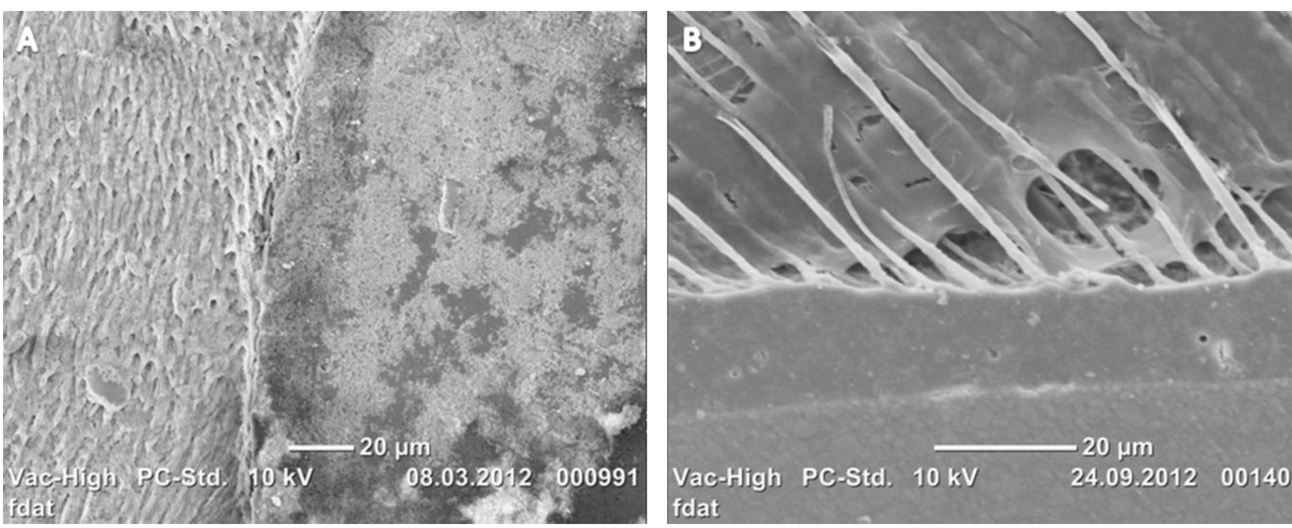

FIGURE 7. Scanning electron microscope image of A, enamel–Optibond All-in-One and B, dentin–Optibond All-in-One interface of control groups.

margins. On the other hand, one-step self-etching adhesives are more prone to water uptake because of their inherent hydrophilicity than the hydrophobic etch-and-rinse adhesives.[42] This way, water from dentin diffuses through the 2-hydroxyethyl methacrylate (HEMA) rich hybrid layers and adhesive layers of these simplified adhesives even after being cured, acting as semipermeable membranes.[43] In HEMA-rich all-in-one adhesives, water reaches the adhesive layer/composite resin interface, forming osmotic blisters of 300 nm to 1.5 µm in diameter,[44] like the ones observed with Clearfil S3 bond.[45] This blister-rich zone jeopardizes copolymerization of monomers from the adhesive and the composite resin resulting in a weaker interface and leading to low bond strength values.[45]

In the present study, etching of enamel and dentin prior the application of Clearfil S3 Bond, Optibond All-in-One, and G-Aenial Bond significantly reduced microleakage scores on enamel margins. Also, etching

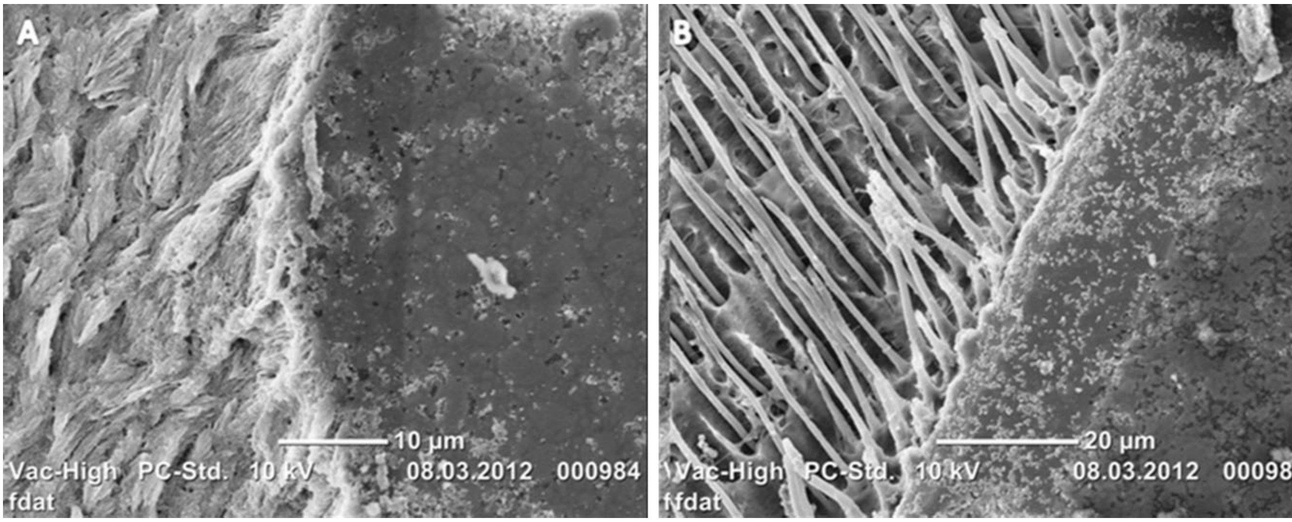

FIGURE 8. Scanning electron microscope image of A, enamel–Optibond All-in-One and B, dentin–Optibond All-in-One interface of prior acid-etching application groups.

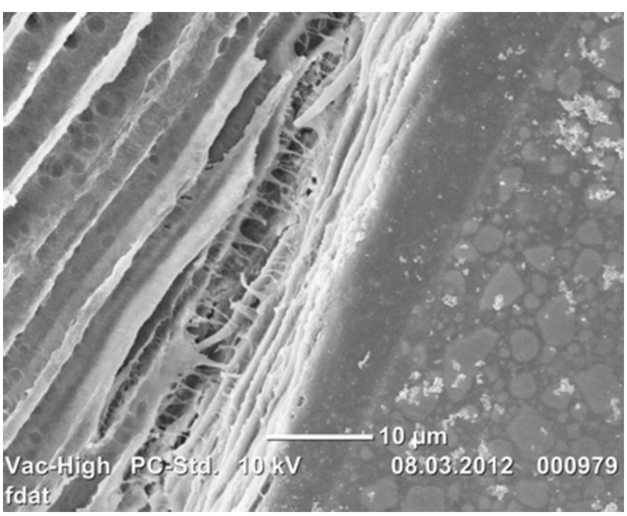

FIGURE 9. Scanning electron microscope image of A, dentin–Optibond All-in-One interface of doubling application time groups.

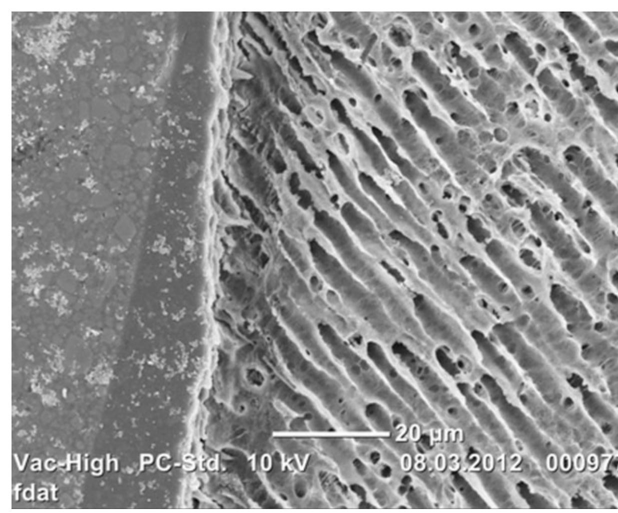

FIGURE 10. Scanning electron microscope image of dentin–Optibond All-in-One interface of two consecutive coats groups.

of the enamel and dentin prior to applying Optibond All-in-One significantly reduced the microleakage compared with the other groups, except rebonding application on enamel margins. On the other hand, etching of enamel and dentin prior to the application of Clearfil S3 Bond exhibited significantly more leakage than G-Aenial Bond and Optibond All-in-One on dentin margins. It was shown that when using one-bottle one-step self-etching adhesives, the margins of restorations in enamel were much worse than the margins of restorations where phosphoric acid was used.[46] Also, it was reported that one-bottle one-step self-etching adhesives were less effective for bonding to enamel; they showed inferior marginal quality scores as compared with the classical etch-and-rinse approach.[46] In addition, it was reported that simplified all-in-one adhesive systems need pre-etching of the margins with phosphoric acid for an effective seal.[47] In agreement with the findings of the present study about leakage of enamel margins, it was reported that when enamel

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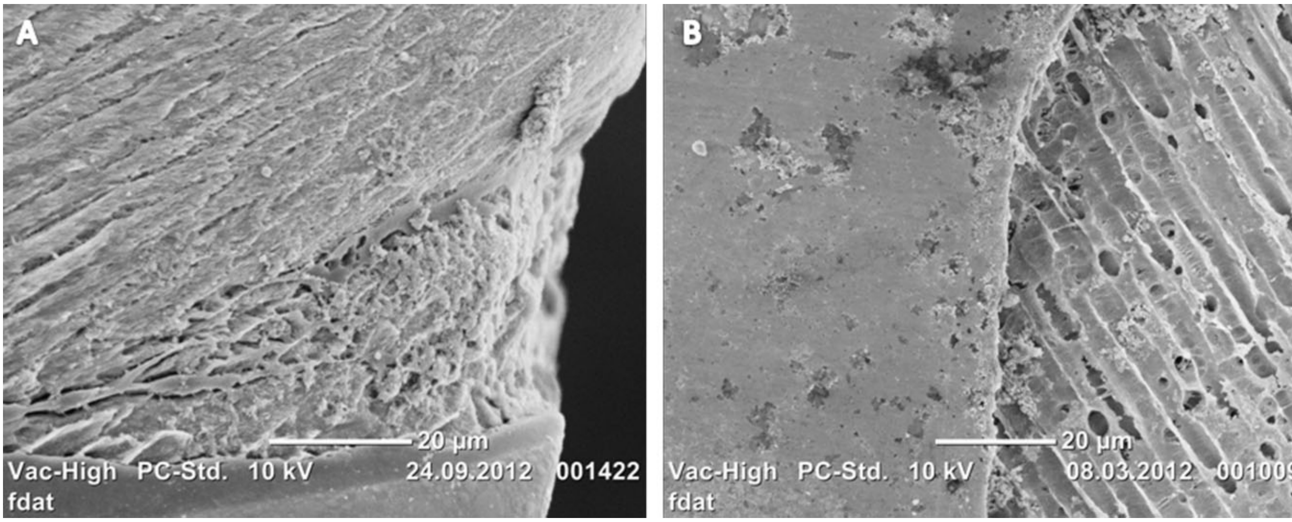

FIGURE 11. Scanning electron microscope image of A, enamel–Optibond All-in-One and B, dentin–Optibond All-in-One interface of rebonding agent application groups.

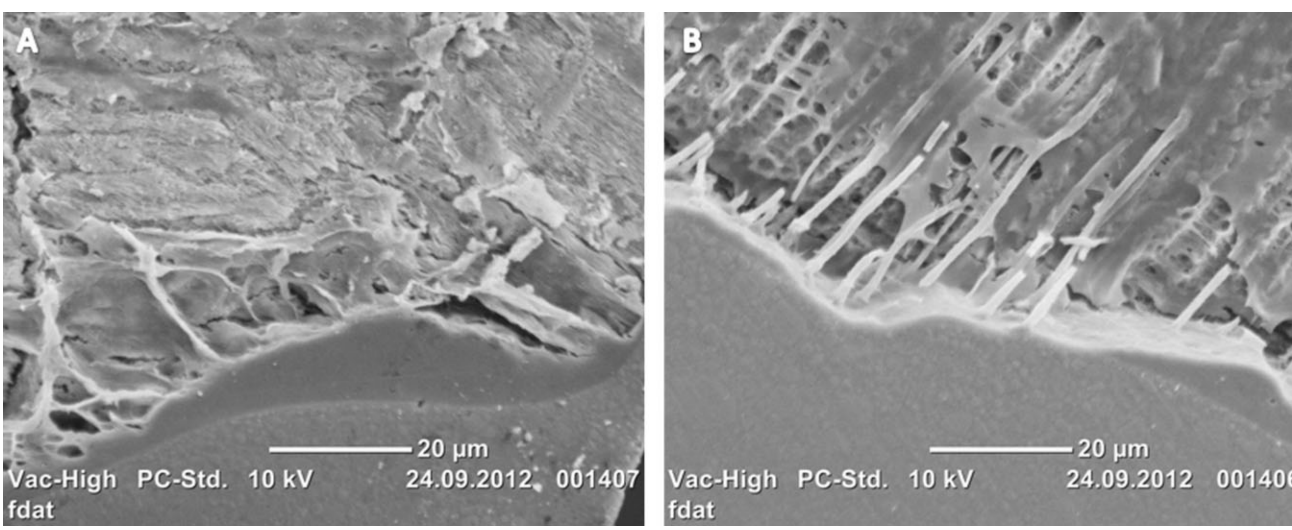

FIGURE 12. Scanning electron microscope image of A, enamel–G-Aenial Bond and B, dentin–G-Aenial Bond interface of control groups.

etching preceded use of the self-etching product, incidence of the marginal leakage of enamel dropped from 0% to 5%, a significant improvement for two-step self-etch adhesive, whereas incidence of dentin margin leakage increased from 55% to 95%, which was not a significant increase for any of the three self-etch adhesives.[48] However, etching of dentin improved the microleakage results on dentin margins for G-Aenial Bond in the present study. Mild self-etch adhesives produce thinner hybrid layers than those produced in total-etch systems.[49] As dentin demineralization is less pronounced, smear plug occludes the orifice of dentinal tubules, which are partially infiltrated by resin; a reduced resin tag formation occurs with these systems.[49–51] Therefore, etching of dentin together with enamel may contribute to the reduction of leakage in the present study.

Doubling the application time of the G-Aenial Bond slightly increased microleakage, which was more than

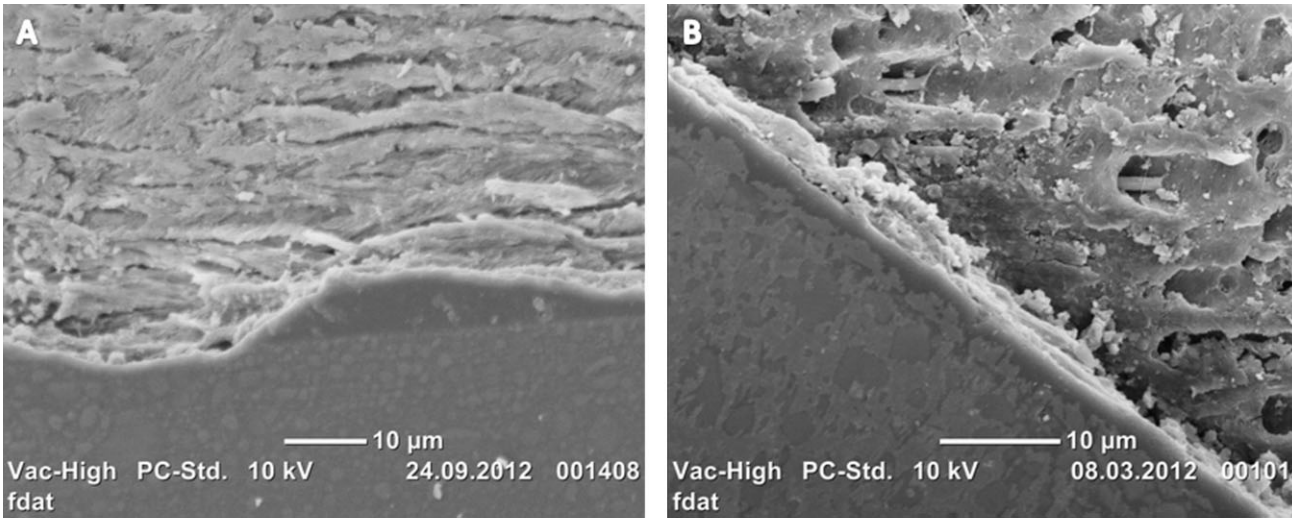

FIGURE 13. Scanning electron microscope image of A, enamel–G-Aenial Bond and B, dentin–G-Aenial Bond interface of prior acid-etching application groups.

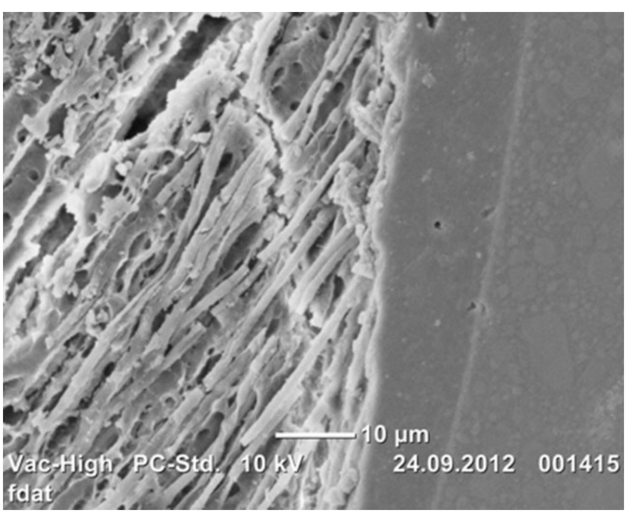

FIGURE 14. Scanning electron microscope image of dentin–G-Aenial Bond interface of doubling application time groups.

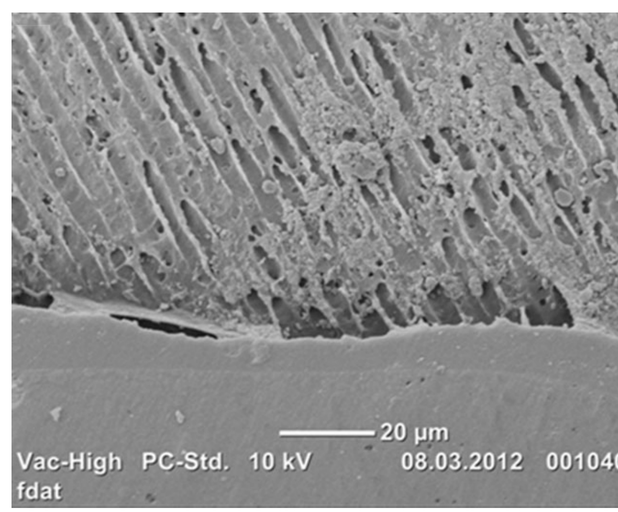

FIGURE 15. Scanning electron microscope image of dentin–G-Aenial Bond interface of two consecutive coats groups.

the control group. On the other hand, etching of the enamel and dentin or rebonding application significantly reduced the microleakage more than the procedure of doubling the application time for G-Aenial Bond on dentin margins. Doubling the application time of Optibond All-in-One caused slightly less leakage than Clearfil S3 Bond on enamel margins. On the other hand, it showed significantly less microleakage than the other two adhesives on dentin margins. It has been reported that when the adhesive is left undisturbed for prolonged periods, a higher amount of solvent could have evaporated, allowing the formation of a stronger polymer within the demineralized dentin and higher resin-dentin bond strengths.[25] However, the water-/ethanol-based system requires a longer application time than the acetone-based system to achieve higher bond strength values. This is probably due to the differences in the vapor pressure among the solvent present in each system.[25] Self-etching systems are very complicated

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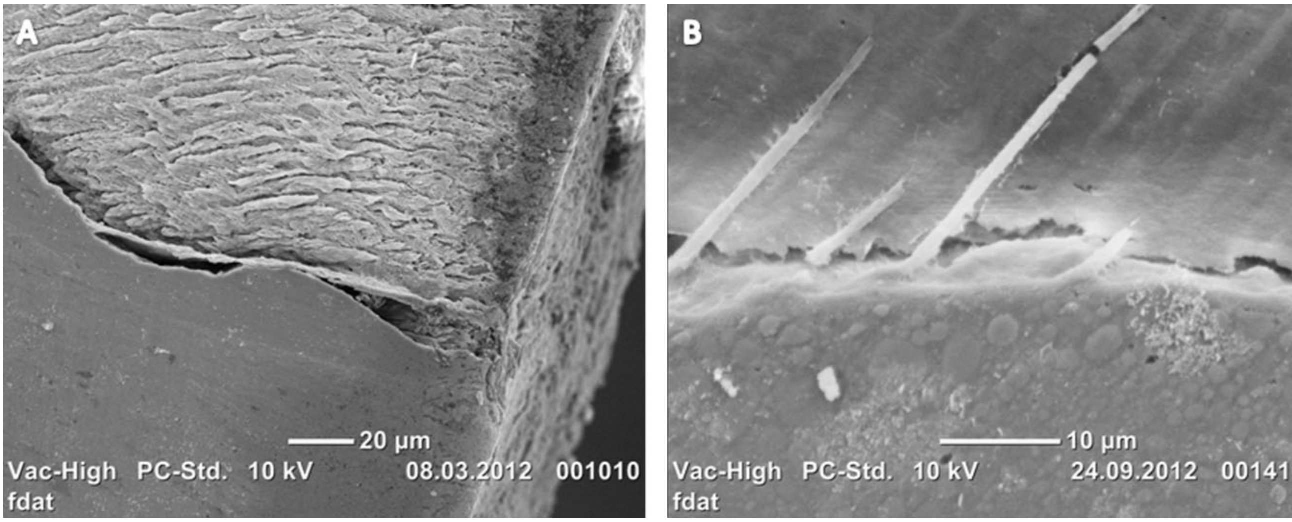

FIGURE 16. Scanning electron microscope image of A, enamel–G-Aenial Bond and B, dentin–G-Aenial Bond interface of rebonding agent application groups.

chemistries.[52] Even though most adhesive systems contain the same components, they may significantly differ, considering the proportional amounts of the ingredients, such as resin, initiator, inhibitor, solvent, and filler particles.[4,53] As a consequence, the particular shortcomings related to the specific compositions of tested adhesive systems might be considered as explaining the different bonding effectiveness obtained with those adhesives.[52]

In this study, applying two consecutive coats of Clearfil S3 Bond significantly improved the microleakage results in dentin margins. Applying two consecutive coats of Optibond All-in-One significantly reduced the microleakage on dentin margins compared with Clearfil S3 Bond and G-Aenial Bond. The presence of water in self-etch adhesives is necessary to ensure the ionization of the acidic monomers, but it is not as efficient as acetone or ethanol as a solvent because of its lower vapor pressure.[54] Conversely, the presence of acetone and ethanol in OptiBond All-in-One might balance the solvent evaporation without dehydrating the dentin because ethanol ensures the wetness of the substrate, and its vapor pressure is intermediate between acetone and water.[55] Another explanation for the good performance of OptiBond All-in-One could be the content of glycerol phosphate dimethacrylate monomer in its formulation, a surfactant monomer that may have facilitated the penetration of hydrophobic components into dentin, reducing the phase separation.[56,57] Also, applying two consecutive coats of G-Aenial Bond exhibited significantly less microleakage than Clearfil S3 Bond in dentin margins. This may be explained by the fact that these application modes may have led to two results: first, it allowed sufficient time for the water to be removed from the first layer, and second, it has possibly allowed sufficient time for a chemical reaction to take place, as this HEMA-free adhesive system contains 4-methacryloxyethyl trimellitic acid (4-MET). This monomer (4-MET) is speculated to have a chemical interaction with hydroxyapaptite crystals.[58,59] It was reported that this led to speculation that the short application time recommended by the manufacturer may not be sufficient to allow the chemical bonding mechanism to take place.[40,58] Similar to the findings of the present study, it has been suggested that the application of multiple coats (two coats and one cure) reduced microleakage scores for a nano-filled acetone-based adhesive system, but an unfilled ethanol-/water-based adhesive system did not give the same result.[38] However, in the present study, applying two coats of dentin adhesives resulted in lower microleakage scores on dentin margins. In Class V cavities, additional adhesive layering in the marginal area reduced the overall degree of the microleakage. The contraction stress generated during the placement of a composite restoration significantly contributes to early microleakage, and this stress was significantly absorbed and relieved by the application of an increasing thickness of low-stiffness adhesive.[26] In addition, it is likely that the increased bond strength seen in both all-in-one adhesives with multiple coatings is due to several mechanisms operating simultaneously.[30] As the first layer of adhesives begins to etch the dentin substrate, it might become rapidly buffered by the hydroxyapatite[60] so that the additional layers of unpolymerized acidic comonomers may improve the etching ability of these adhesives by increasing the concentration of acidic reagents.[61] When using multiple coatings of adhesives, there are several techniques that can be employed. If the solvent is evaporated between each coat, the concentration of comonomers that exists after each coating should increase, thereby facilitating comonomer infiltration with minimal increase in the thickness of the adhesive layer.[30] Moreover, when multiple coats are applied but not cured, the resin infiltration of the hybrid layer and the removal of residual water may be more complete without increasing the thickness of the overlying adhesive layer. When each successive layer is light-cured, the adhesive layer becomes thicker without changing the quality of the hybrid layer.[62]

Rebonding the enamel and dentin margins of the restorations with Clearfil S3 Bond significantly reduced the microleakage when compared with the control group in both margins. Rebonding of the enamel margins for Clearfil S3 Bond significantly reduced the microleakage when compared with Optibond All-in-One and G-Aenial Bond. However, for Optibond All-in-One, rebonding application on enamel margins caused significantly less microleakage than the control and doubling the application time or applying two consecutive coats of this bonding agent. On the other hand, rebonding dentin margins significantly reduced the microleakage compared with the enamel margins for G-Aenial Bond. Also, rebonding dentin margins for Optibond All-in-One and G-Aenial Bond caused significantly less microleakage than the Clearfil S3 Bond. In agreement with the present study results, surface sealants, when applied to Class V composite resin restorations, could contribute to a reduction in microleakage, thus increasing the marginal integrity.[36,63] In contrast, it was also reported that rebonding with a low-viscosity resin did not reduce the microleakage of the restorative systems evaluated in Class V dentin-type cavities.[64] On the other hand, it was indicated that the application of surface sealants promoted reduction in marginal leakage in Class II[65] and Class V cavities.[66–68] However, it was reported that acid-etching prior to rebonding compared with its unetched counterpart increased microleakage in the gingival margins of cavities.[65,66] Whereas in the present study, acid-etching prior to rebonding applied according to manufacturer's recommendations showed some degree of marginal protection (not necessarily always significant). The exposed microdefects and gaps of the subsurface layer might be filled with composite resin smear during finishing procedures. Etching may partially remove the composite resin smear, with some particles being trapped in the bottom of the defects. As a result, the composite surface sealant may show a superficial seal, with surface sealant on the top and smear in the bottom.[65]

This argument is in agreement with SEM observations of the present study. Also, the filler content of the rebonding agent in the present study may contribute to improve the marginal sealing between tooth and restoration, thus avoiding or decreasing the marginal microleakage. In support of this argument, a surface-penetrating sealant with filler as used in the present study demonstrated the lowest immediate microleakage (2%).[67]

CONCLUSIONS

Microleakage was significantly higher on dentin margins compared with the enamel margins for Clearfil S3 Bond in all of the groups. When three dentin adhesives were applied according to the manufacturer's recommendations, Clearfil S3 Bond showed significantly more microleakage than the other adhesives on dentin margins. Etching the enamel and dentin, prior the application of Clearfil S3 Bond, Optibond All-in-One, and G-Aenial Bond significantly reduced microleakage scores on enamel margins. On

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the other hand, etching the enamel and dentin prior to the application of Clearfil S3 Bond significantly increased the microleakage on dentin margins. Doubling the application time of Optibond All-in-One caused significantly less microleakage than the other two dentin bonding agents on dentin margins. Applying two consecutive coats of Optibond All-in-One significantly reduced the microleakage on dentin margins when compared with the results of Clearfil S3 Bond and G-Aenial Bond. Rebonding application on enamel margins for Clearfil S3 Bond and Optibond All-in-One significantly reduced microleakage scores. Rebonding application on dentin margins with Optibond All-in-One and G-Aenial Bond indicated significantly less microleakage than Clearfil S3 Bond. Acid-etching usually promoted reduction of the microleakage in the enamel margins. On the other hand, rebonding application was more effective on the reduction of the microleakage than the other methods in dentin margins.

DISCLOSURE

The authors do not have any financial interest in the companies whose materials are included in the article.

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