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Research Article



In Vitro Evaluation of Microleakage Properties of Etched/Non-Etched Air- Abraded and Conventionally Prepared Cavities: A Scanning Electron Microscopy Study

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In Vitro Evaluation of Microleakage Properties of Etched/Non-Etched Air- Abraded and Conventionally Prepared Cavities: A Scanning Electron Microscopy Study

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Abstract

Statement of the problem: Air-abrasion is an alternative cavity preparation technique using aluminum oxide particles and there is still a lack of microleakage for different cavity forms and restorative materials.

Objective: The aim of the study is to evaluate microleakage of etched/non-etched cavities prepared with air- abrasion and conventional technique using light and scanning electron microscopy.

Materials & Methods: 69 non-carious human molars were selected and randomly allocated to 9 groups: Group 1 (G1), Group 2 (G2), Group 3 (G3), Group 4 (G 4), Group 5A (G5A), Group 5B (G5B), Group 6A (G6A), Group 6B (G6B), Group 7 (G7). Class I cavities were prepared on the G1, G2, G5A, and G5B samples with air-abrasion technique whereas conventional method was used on the samples of G3, G4, G6A, and G6B groups. G7 was assigned as the control group. Adhesive and composite were applied without using acid etching in G1 and G3. G2 and G4 samples were acid etched prior to the bonding and composite application. The cavities of G5A and G6A samples were filled with temporary filling material. The G5B and G6B samples were not restored. No preparation or restoration was performed on G7 group teeth. All samples were stored in distilled water at room temperature until thermocycling. Then the samples were immersed in 50% diluted indian ink at 37°C for 48 hours prior to the sectioning, microscopic evaluation and, assessment of dye penetration. The Kruskal Wallis and Mann Withney U test were used for comparisons of the obtained data ($\alpha = .05$ for all tests)

Results: Statistically significant differences were found between Group 1 and 2, Group 1 and 3, Group 1 and 4, Group 2 and 3, Group 2 and 4, Group 3 and 4 ($P < .05$).

Conclusion: Due to the results of this study, acid etching is essential for the restoration of air-abraded and conventionally prepared cavities to prevent microleakage.

Keywords: Cavity Preparation Techniques, Conventional Cavity Preparation, Air-Abrasion, Acid Etching, Microleakage, Scanning Electron Microscopy.

Introduction

Cavity preparation techniques were first introduced by Black¹ in 1893, and used for direct restorations for a long time. “Extension for prevention” was one of the main principles; extension cavity margins in order to prevent seconder carious lesions. The basis of this technique is using rotary drills to prepare the cavity in a specific geometry and retentive form to enhance the restoration longevity.

During late 1950’s studies and developments in adhesive techniques changed the cavity preparation techniques.²⁻⁶ One of the most important changes was the minimally invasive technique (MIT).^{7,8} The basis of MIT is “less is more”; less preparation is more strength and longevity.⁹⁻¹¹

Due to the disadvantages of rotary drills alternative cavity preparation techniques were developed in 1943.⁴ Air- abrasion technique, which is basically spraying the tooth surface with high velocity particles, is one of the alternative techniques.^{12,13} Air-abrasion generates no heat, vibration, or pain.¹⁴ Also there is no discomforting noise. Adhesive materials can be used in the cavities prepared with air-abrasion because there is less tooth loss in this technique.^{8,15,16}

The success of a restoration depends on the adaptation to cavity walls. However, each restorative material permits passage of fluids and micro-nutrients.¹⁷ The passage of bacteria, fluids, molecules and ions between the cavity wall and the restorative material which is not determined clinically is called microleakage.¹⁷

The aim of this study is to evaluate and compare the microleakage of the composite restorations with/without acid etching on Class I cavities prepared with air abrasion and air turbine under light and scanning electron microscope.

Null hypothesis is acid etching reduces microleakage in both air-abraded and conventionally prepared cavities.

Materials & Methods

The present study was approved by the Ethical Committee of Ege University Faculty of Dentistry, and executed in accordance with the Helsinki Declaration of 1964 as revised in 2013 in Brasil.

72 periodontally extracted molar teeth were collected and each tooth was cleaned using wire brush, and toothbrush for 3 minutes respectively. Teeth were rinsed under tap water for 5 minutes and stored in a polycarbonate cup full of distilled water at room temperature. No anti-bacterial agent was used in cleaning procedures. Before cavity preparations, an experienced endodontist examined the teeth; 3 teeth were found unsuitable for the study and excluded. Thus, 69 non-carious human molars were randomly allocated to 9 experimental groups: Group 1 (G1), Group 2 (G2), Group 3 (G3), Group 4 (G 4), Group 5A (G5A), Group 5B (G5B), Group 6A (G6A), Group 6B (G6B), Group 7 (G7). The procedures applied in each experimental group and the number of specimens included are given below.

G1 (n = 11): Class I cavities were prepared with air abrasion technique using 25 µm aluminum oxide particles at 120 psi, 0.016-inch nozzle tip, and 4 mm nozzle tip distance with an angle of 90° to the enamel surface. The cavity preparation depth and width were 3mm and 1/3 of the intercuspal distance respectively.

The prepared cavities were rinsed with two- in- one syringe and dried with oil free air. Bonding agent (Scotchbond Multipurpose Plus Adhesive System, 3M Dental Products, USA) was applied and cured according to the manufacturer's instructions. Valux Plus Composite Restorative material (3M Dental Products, USA) was placed by using incremental layering technique and each layer was cured by Degulux Light Curing Unit (Degussa AG, Germany) for 60 seconds.

G2 (n = 11): Cavities were prepared using air-abrasion technique with the same specifications. The cavities were rinsed with two- in- one- syringe and dried with oil free air. All cavities were etched with %35 phosphoric acid gel (3M Dental Products, USA). Enamel was etched for 30 seconds while dentine was etched for 15 seconds. The teeth were then thoroughly rinsed for 30 seconds and dried with oil free air having leaving the dentine slightly moist. Scotchbond Multipurpose Plus Adhesive system was applied to the cavities according to the manufacturer's instructions. After adhesive application Valux Plus Composite Restorative material was placed by using incremental layering technique and each layer was cured again by Degulux Light Curing Unit for 60 seconds.

G3 (n = 11): Cavity preparations were performed with a No 801/014 bur at high speed with water coolant, the bur was replaced after every third preparation. After cavities were rinsed with two- in- one syringe and dried with oil free air, Scotchbond Multipurpose Plus Adhesive System was applied to the cavities according to the manufacturer's instructions. Valux Plus

Composite Restorative material was placed by using incremental layer technique and each layer was cured by Degulux Light Curing Unit for 60 seconds.

G4 (n = 11): the same cavities were prepared with a No 801/014 bur at high speed with water coolant; the bur was replaced after every third preparation. The prepared cavities were rinsed with two- in- one syringe and dried with oil free air. All cavities were etched with %35 phosphoric acid (3M Dental Products, USA). The enamel was etched for 30 seconds and the dentin was etched for 15 seconds. The teeth were then thoroughly rinsed for 30 seconds and dried with oil free air having slightly moist dentin. was applied to the cavities according to the manufacturer's instructions. After adhesive application Valux Plus Composite Restorative material was placed by using incremental layer technique and each layer was cured again by Degulux Light Curing Unit for 60 seconds.

G5A (n = 5): Class I cavities were prepared with air abrasion technique using 25 µm aluminum oxide particules at 120 psi using 0.016 inch nozzle tip and nozzle tip distance was 4 mm with an angle of 90° to the enamel surface. The cavity preparation depth was 3mm and 1/3 of the intercuspal distance.

The prepared cavities were rinsed with two- in- one syringe and dried with oil free air. The prepared cavities were restored with a temporary filling material (Cavit®, ESPE Premiere Inc., USA).

G5B (n = 5): The cavities were again prepared using air abrasion technique at the same conditions. The prepared cavities were rinsed with two- in- one syringe and dried with oil free air. The prepared cavities were not restored with any restorative material.

G6A (n = 5): The cavities were prepared with a No 801/014 bur at high speed with water coolant; the bur was replaced after every third preparation. The prepared cavities were rinsed with two- in- one syringe and dried with oil free air. The prepared cavities were restored with temporary filling material.

G6B (n = 5): The cavities were prepared with a No 801/014 bur at high speed with water coolant; the bur was replaced after every third preparation. The prepared cavities were rinsed with two- in- one syringe and dried with oil free air. The prepared cavities were not restored with any restorative material.

G7 (n = 5): No cavities were prepared.

All samples of the study groups were stored in distilled water at room temperature following the procedures. After 10 days, each of the 69 samples were thermocycled 500 times. A cycle was consisted of 40 seconds in each water bath and an exchange time of 13 seconds between baths.¹⁸ 1st bath water temperature was $5 \pm 5^{\circ}\text{C}$, whereas 2nd one was $55 \pm 5^{\circ}\text{C}$. After thermocycling, the specimens were dried and prepared for testing for microleakage. The roots and apices were sealed with sticky wax. The specimens were coated with two coats of nail varnish to prevent dye penetration except for the restoration site and 1 mm rim of the tooth structure surrounding the restoration. Then the specimens were immersed in %50 diluted indian ink (Pelikan, Germany) at 37°C for 48 hours.

After 48 hours, the specimens were rinsed with tap water and prepared for sectioning. Roots were removed at the cement- enamel junction and the crowns were sectioned mesiodistally with a low speed diamond saw with water coolant. Each specimen was examined by a reflecting light microscope (Baush & Laumb, 1.5x, Canada) by three independent evaluators and any discrepancies were discussed. Both halves of each specimen were examined and scored. The score for each tooth was mean of these scores. The following scale was used for microleakage examination; 0: no leakage, 1: penetration of half distance to the axial wall, 2: penetration to cavity base, 3: penetration involving cavity base, 4: penetration of pulp chamber.

In addition, two specimens were selected randomly from G1, G2, G3, G4. The specimens were embedded in epoxy resin and sectioned mesiodistally with a low-speed diamond saw with water coolant. The sectioned surfaces of the specimens were wet ground with P360A, P400A, P800A and P1000A silicon carbide paper, respectively. Then the surfaces were etched using %35 phosphoric acid gel for 10 seconds in order to remove smear layer. The specimens were then prepared to examine the coherency between composite restorative material and cavity by scanning electron microscope (SEM) (Jeol JSM 5200, Japan)

The mean scores of mesial and distal halves were evaluated for statistical analysis of microleakage scores. The data were analyzed by Kruskal Wallis, Mann-Whitney U test and Bonferroni correction.

Results

Microleakage scores of mesial and distal surfaces of the 69 specimens from 9 different groups assessed by reflecting light microscope are given in Table 1 and 2.

Table 1. Microleakage scores of mesial surfaces of specimens of 9 study group.

	N	Microleakage Scores				
		0	1	2	3	4
G1	11				3	8
G2	11	5	6			
G3	11			6	5	
G4	11	1	4	6		
G5A	5		3	2		
G5B	5					5
G6A	5			5		
G6B	5					5
G7	5	5				

Table 2. Microleakage scores of distal surfaces of specimens of 9 study group.

	N	Microleakage Scores				
		0	1	2	3	4
G1	11				1	10
G2	11	4	5	2		
G3	11			6	5	
G4	11	2	2	7		
G5A	5		4	1		
G5B	5					5
G6A	5			2	3	
G6B	5					5
G7	5	5				

In G1, 2 of the specimens with microleakage score of ‘4’ were randomly selected and subjected to SEM analysis. Significant detachments between dentin and adhesive material were observed (Figure 1). Although composite resin- adhesive attachment was uniform, adhesive-enamel attachment was partly in poor shape (Figure 2). Gaps were increased especially due to voids of composite resin in the cavity margins.

Figure 1. View of the uniform attachment between composite resin and adhesive materials.

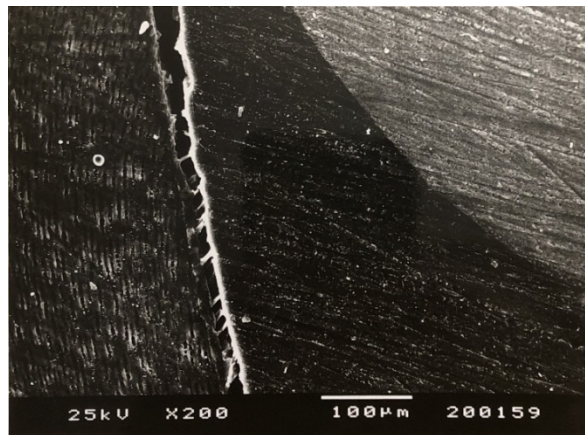
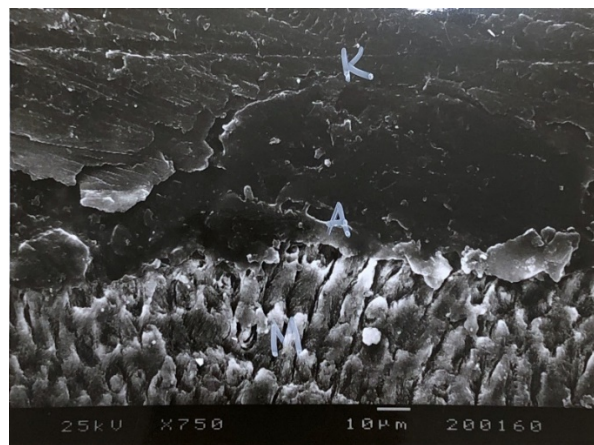


Figure 2. View of partly bad connection between adhesive and enamel.

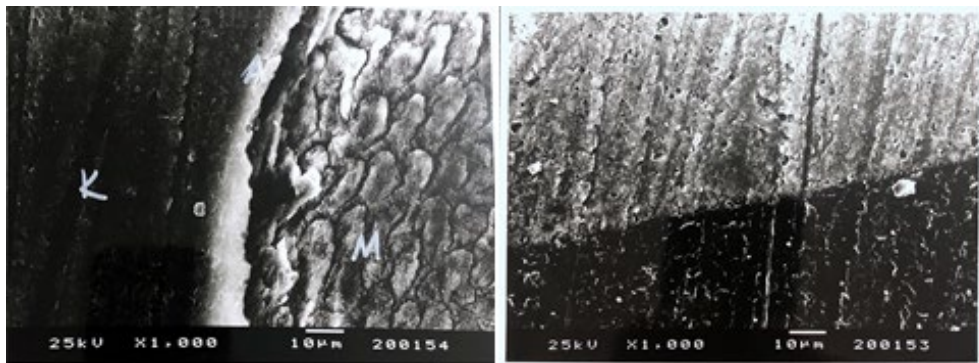


SEM investigation of the specimens of G1 and G2 showed that cavity adaptation of filling material was satisfactory and there was no adhesive accumulated in cavity corners (Figure 3). Also, no gaps between composite and enamel and composite and dentin were observed (Figure 4).

Figure 3. View of the specimens which have no accumulation of adhesive in cavity corners.



Figure 4. View of the adaptation of composite to enamel and dentin.



SEM investigation of the specimens of the G3 revealed that after desiccation, filling material and dentin were detached from cavity base and cohesive failures occurred in dentin. Also adhesive accumulation was seen in cavity corners. However, cavity adaptation of composite was exceptional and there was no detachment (Figure 5). There was a slight gap and in this gap resin tags were seen in another specimen of G3 (Figure 6). Cohesive failures were observed inside enamel, dentin, and composite, whereas composite- adhesive attachment was intact.

Figure 5. View of the cavity adaptation of composite resin material.

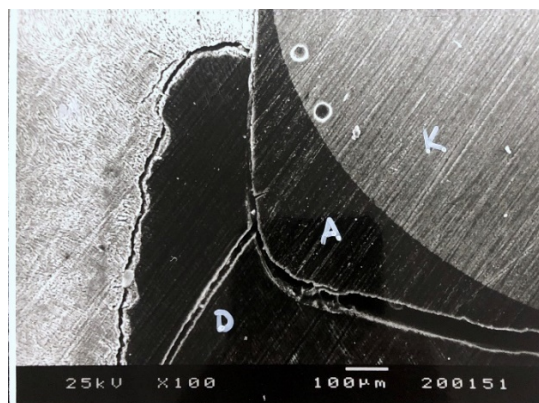
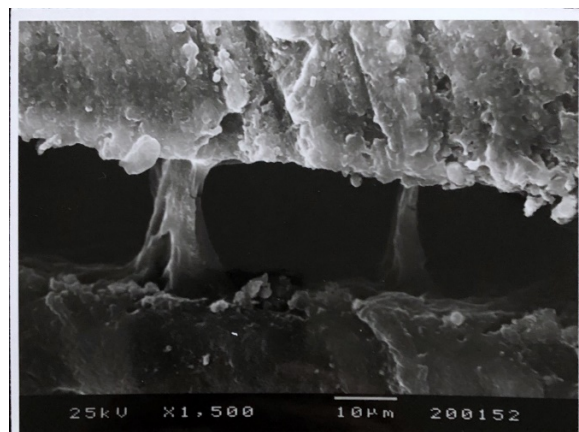
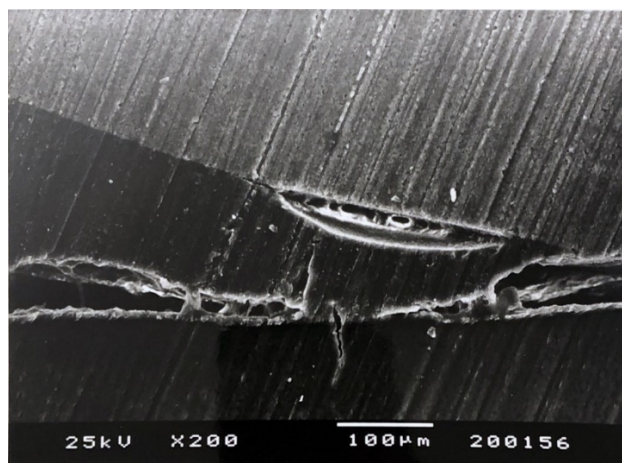


Figure 6. View of the slight gap and resin tags seen in the gap.



Cohesive failures were observed in composite and dentin in G4 specimen despite the existing resin. (Figure 7).

Figure 7. View of the adhesive failure between dentin and composite resin material.



Descriptive analysis results of microleakage scores of 8 groups are given in Table 3.

Table 3. The mean and standard deviation values of microleakage scores obtained from 8 study groups.

	Mean Values and Standard Deviations
G1	3,9 ± 0,3
G2	0,9 ± 0,7
G3	2,5 ± 0,5
G4	1,6 ± 0,7
G5A	1,4 ± 0,5
G5B	4,0
G6A	2,0
G6B	4,0

Differences between groups were analyzed by Kruskal Wallis and Mann Withney U tests ($\alpha = .05$ for all tests) in terms of significance. The P values obtained from the two comparisons are given in Table 4.

Table 4. The results of Mann Withney U test.

	G1	G2	G3	G4
G1		$P < .05$	$P < .05$	$P < .05$
G2	$P < .05$		$P < .05$	$P < .05$
G3	$P < .05$	$P < .05$		$P < .05$
G4	$P < .05$	$P < .05$	$P < .05$	

Discussion

Within the limitations of this study, it was observed that acid etching reduced microleakage thus, the null hypothesis for this study was accepted.

The results of this study on naturel teeth are similar to the studies analyzing relation between microleakage, air-abrasion, and acid etching. It is well documented that the microleakage around a restoration might cause pulpal inflammation beside marginal discoloration, secondary caries, and bacterial penetration. The smear layer formed on the dentin surface during cavity preparation prevents bacteria from reaching the pulp. Fusayama¹³ suggested to open the dentin tubules by etching the dentin to form a rough intertubular layer. Thus, when the adhesive is bonded to the acid-treated dentin, the restoration margins would be sealed and prevent bacterial leakage and subsequent pulpal irritation.^{13,19}

To date, no restoration material with perfect adaptation/adhesion to the tooth has been developed.

Microleakage occurs due to many factors. These factors include dimensional change of the restoration, polymerization and thermal shrinkage, fluid absorption, mechanical stress, and dimensional change of the tooth.¹⁷

As a result of microleakage, problems such as cracks and fractures occur in restorative materials that shorten the life of the restoration. Many methods are used to detect microleakage: staining with different dyes, SEM, chemical agents, neutron activation analysis, ionization, autoradiography, radioisotope, reverse radioactive absorption.¹⁷

The term nanoleakage expresses “fluid transport along with resins at the enamel- dentin boundary with restoration” and can only be detected by advanced SEM techniques.¹⁷

Although manufacturers claim that there is no need for acid etching on air-abraded surfaces, many studies, especially since the mid-1990s, have reported the opposite. In 1995, Eakle et al²⁰ stated that the least leakage was in the air-abrasion and acid-applied group among the Class 1 cavities prepared by air-abrasion and conventional methods, in which 27 µm aluminum oxide and 120 psi pressure were used. Furthermore, no significant difference was found between the air-abrasion and acid-applied groups and the conventional and acid-applied groups. It was also reported that although microabrasion roughened the surface, microleakage was ex-

cessive without acid etch.^{7, 21} In a similar study, Davis et al²¹ stated that microleakage differences were significantly greater in the sealants performed without acid application compared to those acid etched.

Guirguis et al²¹ also found that microleakage was significantly higher in restorations without acid etch application in the cavities air-abraded with 50µm particle size aluminum oxide and 120 psi pressure.

Contrary to results of these studies, Keen et al²² stated that microleakage was similar in specimens prepared by air-abrasion and conventional methods. Wright et al²³ also conducted a study similar to Keen et al. (22), and stated that there was no statistically significant difference in the microleakage of teeth acid etched or only air-abraded before sealant application.^{22, 23}

In our study, the least amount of microleakage, which was statistically significant, was detected in air-abraded + acid etched specimens (mean value 0.9 ± 0.7). Statistically significant differences were found between this group, and the only air-abrasion group, the conventionally prepared and conventionally prepared + acid etched groups ($P < .05$). Following the air-abraded + acid etched group, the lowest microleakage was obtained from the conventionally prepared + acid etched group specimens. The highest microleakage was observed in the group where only air-abrasion was applied (mean value 3.9 ± 0.3). According to these findings, it may be stated that acid etching of the cavity walls after air-abrasion application reduces microleakage. The reason of the high microleakage in the groups where just air-abrasion is applied may be the smear layer and the debris in the cavity. It is thought that although the cavities were thoroughly rinsed with air-water spray, the debris in the cavity cannot be removed sufficiently. However, debris and smear layer in the air-abrasion and acid etch group were eliminated by the application of 35% phosphoric acid.

Guirguis et al²¹ suggested that when compared to acid etch, air-abrasion alone does not provide sufficient sealing, and a possible explanation for this may be different etching protocols or the formation of a different number of resin tags in each method. They stated that the number of resin tags formed and the quality of the enamel/resin interface affect microleakage. For this purpose, Brockmann et al²⁴ investigated the effects of enamel air-abrasion application by examining the shape and number of formed resin tags. They also compared the bond strength of sealants applied to air-abraded enamel. In these studies, they compared air-abrasion and conventional methods with and without acid-etch, and they stated that air-abrasion alone makes a smooth surface that creates less resin tag. This indicates that they have significantly lower shear

bond strength resistance with air-abrasion. The more resin tags formed, the higher their bonding capacity.²¹

Hannig and Femerling²⁵ investigated the effects of air-abrasion on the bonding between composite and dentin, and they stated that the combination of air-abrasion/dentin binding effectively reduces the formation of space at the composite resin-dentin interface. They stated that air-abrasion could not replace the dentin adhesives required for bonding the composite to the dentin surface. Roeder et al⁵ also obtained similar results. Prati et al²⁶, Yu et al²⁷, Gwinnett²⁸ and Kanca²⁹, Nakabayashi and Saimi³⁰ stated that the smear layer formed during cavity preparation reduces the effectiveness of dentin bonding agents. Pashley et al³¹, Nakabayashi, and Saimi³⁰ indicated that intertubular dentine resin infiltration and hybrid layer formation is easier on the fractured dentin surface than on the dentin surface covered with the smear layer. Air-abraded dentin surfaces can only be covered with a smear layer, so they have more favorable conditions for bonding to dentin than non-air-abraded, smear-coated dentin surfaces.²⁵

Haws et al³², Zyskind et al³³ stated that acid etch application reduces microleakage following air abrasion. In the study of Freeman et al³⁴, it was observed that while the adhesion to the dentine was sufficient in the cavities prepared with the conventional method in which self-etch adhesives were applied, the number of resin tags was relatively low. In the same study, resin tags were increased in cavities where air-abrasion and acid etch were applied. Despite the increase in the amount, length, and diameter of the resin tag in the cavities prepared with air-abrasion, unfortunately, the hybrid layer on the dentin surface has many defects, and it increases the shear bond resistance in a small amount.

We obtained similar results with Hannig and Femerling²⁵ in SEM examinations of the specimens prepared with air-abrasion. It was observed that the bonding between the composite and the adhesive layer was substantial. Also, cavity adaptations of the restorations on the teeth of acid etched air abrasion group were found remarkably superior to the non-etched specimens. Since no gap between the cavity and the restoration material was detected, no resin tags were seen in air abrasion specimens. Assessment of the specimens prepared with the conventional method also revealed that despite the exceptional cavity adaptation there were cohesive ruptures in enamel, dentin, and composite. Resin tags were seen through the gap between the dentin and the restoration. Cohesive failures and resin tags were also observed in the conventionally prepared and acid-etched specimens. In the specimens of this group, a perfect adaptation was observed between composite-adhesive and enamel-adhesive connections.

According to the results of this study, the incidence of marginal spacing and microleakage decreases in case of acid etching application to the cavity surfaces prepared with air abrasion. In addition, it can be stated that the microleakage detected in the not etched air-abrasion group specimens was caused by the gap between the cavity walls and the restoration.

Conclusion

Within the limitations of this study, it is concluded that acid etching is an essential factor in establishing successful bonding and cavity adaptation. Furthermore, etching decreases microleakage for all preparation techniques.

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