AJDS ALAZHAR Al-Azhar Journal of Dental Science
Vol. 26- No. 4- 563:572- October 2023
Print ISSN 1110-6751 | online ISSN 2682 - 3314
https://ajdsm.journals.ekb.eg
Orthodontic & Pediatric Dentistry Issue (Orthodontics and Pediatric Dentistry)

EFFECT OF TWO DIFFERENT INVASIVE TOOTH SURFACE PREPARATION TECHNIQUES ON PENETRATION, MARGINAL ADAPTATION, AND RETENTION OF PIT AND FISSURE SEALANT (AN IN VITRO STUDY)

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ABSTRACT

Objectives: the present study was conducted to evaluate the effect of Two Different Invasive Tooth Surface Preparation Techniques on Penetration, Marginal Adaptation, and Retention of Pit and Fissure Sealant. **Subjects and methods**: A total of 60 permanent mandibular molars were divided into five equal main groups according to the pits and fissure preparation protocol as follow: Group A: Acid-etching, Group B: Air abrasion, Group C: Ultrasonic preparation, Group D: Air abrasion followed by acid-etching preparation, and Group E: Ultrasonic followed by acid-etching preparation. Measurements of sealant marginal adaptation shear bond strength, and resin penetration. **Results**: air abrasion or ultrasonic followed by acid etching, and acid etching alone exhibited less microleakage. Acid-etching alone. Ultrasonic alone resulted in a significant increase in the resin retention when compared with air abrasion alone. However, the use of ultrasonic in combination with acid etch resulted in an insignificant increase in the resin retention when compared with air abrasion in combination with acid etch. **Conclusion:** acid etching remains the most effective and simplest technique in sealants' success. The use of air abrasion followed by acid etching can be recommended to maintain an adequate seal.

KEYWORDS: Pit and Fissure Sealant, Penetration, Marginal Adaptation, and Retention.

INTRODUCTION

Dental caries is a multifactorial disease caused by the host, agent, and environmental factors. Streptococci Mutans (S. mutans) is the primary etiologic agent of dental caries. Through adhesion, S. mutans attaches to the dental pellicle and breaks down sugars for energy to produce lactic acid, causing an acidic environment around the tooth. As a result, demineralization of the enamel and, subsequently, the dentin occurs [(1)].

The majority of dental caries in young children occur in pits and fissures. Pits and fissures are more susceptible to dental caries because the anatomy favors plaque accumulation; these areas are often too narrow for any oral hygiene measures to be effective. By filling such irregularities with

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DOI: *10.21608/AJDSM.2022.151234.1350*

flowable restorative material, the area becomes less morphologically susceptible [2].

Modern procedures focus a higher emphasis on disease prevention and tooth structure preservation as a result of the shift from a surgical to a medical approach to disease therapy. Oral hygiene is one of them (daily plaque removal by brushing, flossing, and rinsing is one of the best ways to prevent dental caries), Fluoride (fluoride prevents dental caries by inhibiting demineralization of the crystal structures inside the tooth and enhancing remineralization), Xylitol (sucrose is a well-known cause of dental caries, and higher sucrose intake increases the risk of dental caries), Vaccine, and Pit and Fissure Sealants [3].

Pits and fissures sealants can protect the occlusal tooth surface by providing a smooth and clean surface, decreasing the food retention, as well as it can reduce bacterial colonization and growth. Therefore, the pits and fissures sealants have a significant role in caries prevention, and they isolate pits and fissures from the bacteria and their by-products provide a mechanical barrier and avoid the accumulation of dental plaque. Therefore, Pits and fissures sealants application is one of the most reliable and effective methods for preventing occlusal caries. The advantage of the sealant application is significant caries risk reduction compared to non-sealed controls and lower cost compared to restoration placement [4].The preventive properties of PFS can only be guaranteed if the restoration is retained in its entirety [5].

While using a standard acid conditioning regimen is a common technique in surface preparation, it has a number of disadvantages, including the partial eradication of organic residues and a decrease in enamel susceptibility to caries due to demineralization [6].The Air abrasion technique can be used as an alternative method for acid etching as it utilizes abrasive particles, such as alumina (Al2O3), to strike the tooth at high air pressure. Despite the number of scientific works on pit and fissure surfaces preparation techniques, very little is known about the use of ultrasound tips for this purpose. The most common use of ultrasound in dentistry is for professional oral hygiene, to clean dental surfaces from plaque and calculus, or in endodontic root canal treatment. For this intention, high-intensity and low-frequency ultrasound are used [7]. Therefore, the present study was intended to evaluate the effect of two pits and fissures preparations techniques on the efficacy of pit and fissure sealant.

SUBJECTS AND METHODS

Study Design:

This study was designed as an in vitro experimental study.

Study Setting:

Measurements were carried out at a private lab using scanning electron microscopy at the Egyptian Atomic Energy Authority (EAEA).

Inclusion criteria:

- Freshly extracted mandibular permanent molars.
- Permanent molars with deep pits and fissures.
- Sound teeth free from any decay or restoration (dental caries were detected by visible color and texture change, tactile sensation using a dental explorer).
- Teeth free of cracks and developmental defects (UV; light cure).

Exclusion criteria:

- Carious or previously restored teeth.
- Teeth with previous pits and fissure sealant.
- Teeth with macroscopic fractures or attrition.
- Developmental defects as enamel hypoplasia.
- Teeth with macro-cracks.

Sample size:

Based on previously treated trial cases [8], we conducted a power analysis (G power version 3.1 statistical software, Franz Faul, Universität Kiel Germany). The input parameters were α error probability of 0.05, an effect size (f) of 0.48, and a power of %80. The findings indicated a minimum sample size of n = 60 samples, (12 samples for each group).

Ethical Consideration:

This work was approved by the Ethical Committee of the Faculty of Dental Medicine, Al-Azhar University (Boys, Cairo), with the permission number EC Ref. No. (538/3043)

Sample grouping:

A total of 60 permanent mandibular molars (n=12 for each group) were used during this study. The teeth were randomly divided into five equal main groups according to the pits and fissure preparation protocol as follow: Group A; occlusal pit-and-fissure pretreated with acid etch alone (control group), Group B; occlusal pit-and-fissure pretreated with air abrasion alone, Group C; occlusal pit-and-fissure pretreated with ultrasonic alone, Group D: occlusal pit-and-fissure pretreated with air abrasion followed by acid etch; and Group E: occlusal pit-and-fissure pretreated with ultrasonic followed by acid etching. Then, each main group was subdivided into three equal subgroups (n=4) according to the type of test (resin penetration, marginal adaptation, and retention).

Sample Preparation Fig (1):

1. Teeth Selection and Cleaning:

- A freshly extracted mandibular permanent molar free from cracks, caries, or restoration was used.
- Removal of soft tissue remnants and calculus, the occlusal surfaces of all teeth were cleaned.
- Subsequently, the teeth roots were cut off 2 mm below the cementoenamel junction (CEJ) and placed in distilled water at 4°C until use for no longer than two weeks [2].

2. Pit and Fissure Preparation:

Pits and fissures of the occlusal surfaces of each main group were prepared by the different surface preparation protocols as follow: -

Acid Etching Surface Preparation Fig (1)**:**

For the teeth of (group A); the Occlusal pit and fissures enamel of each tooth was conditioned for 30 seconds with 37% orthophosphoric acid gel (3M ESPE Scotchbond Universal etchant) then rinsing by water.

Air Abrasion Surface Preparation:

For the teeth of (Group B); the occlusal fissures were prepared with air abrasion, water spray aluminum oxide sandblaster system (Air abrasion Master, Foshan, Guangdong, China) using 50 mm alumina particles.

Ultrasonic Surface Preparation:

For the teeth of (Group C) the fissures were treated with an ultrasound tip (T1) mounted on a handpiece activated by the ultrasonic device (Woodpecker, UDS-A LED, Guilin Woodpecker Medical Instrument, China) ultrasonic instrument.

Combination Groups:

For the teeth of (Group D); following the air abrasion surface preparation, the occlusal fissures of the teeth were rinsed, dried, and etched with 37% orthophosphoric acid gel for 30 seconds, and then rinsed by water and dried as mentioned. For the teeth of (Group E); following the ultrasonic surface preparation, the occlusal fissures of the teeth were rinsed, dried, and etched with 37% orthophosphoric acid gel for 30 seconds, and then rinsed by water and dried for 30 seconds.

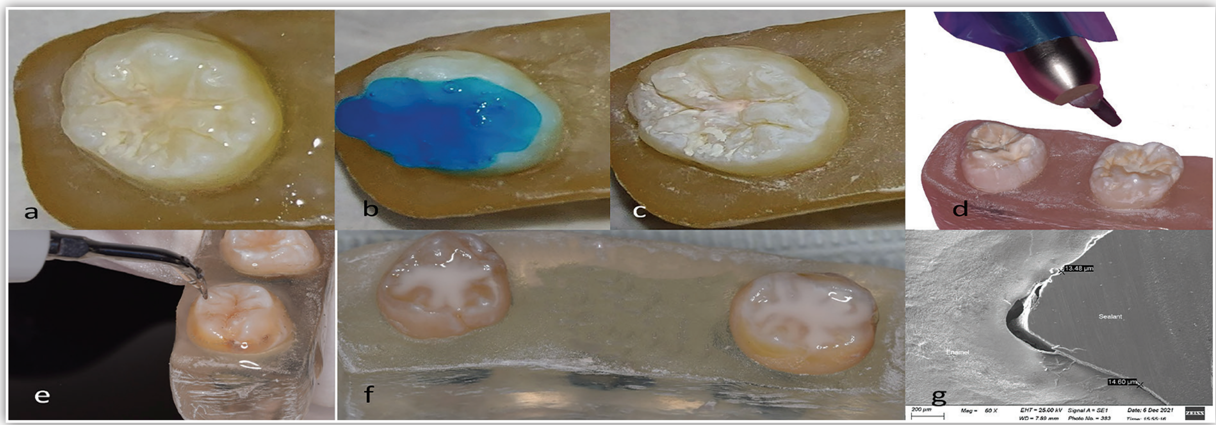

FIG (1) a-c, Acid conditioning of pit and fissures show Chalky white prepared occlusal surface, d, teeth preparation with Air abrasion, e, teeth preparation with ultrasonic, f, Sealant application, and g, depth of resin penetration

Sealant Application Fig (1):

For all specimens in all groups, the resin-based pit and fissure sealant was applied to the surface directly by small tip. Then, the sealant was cured using an LED light (Monitex BlueLex 105, Monitex Industrial Co., Garden City, Idaho, USA) curing unit for 20 seconds. The specimens of all groups were then incubated for 24 hours in distilled water at room temperature [9].

Testing Procedures:

1. Evaluation of resin penetration:

All specimens were buccolingually sectioned into three fragments by high-speed precision saw (Isomet 4000, Buehler, Evanston, IL, USA).

Then, the sectioned samples in each group the depth of resin penetration were detected using scanning electron microscopy (ZEISS- EVO 15- UK).

2. Evaluation of marginal adaptation:

- For this test teeth were stored for 4 weeks in distilled water, and then the teeth were thermocycled in a water bath thermocycling device (JULABO, FT200 immersion cooler, USA) between 5°C and 55°C for 500 cycles [9].
- Each tooth was mounted in self-curing acrylic resin block (Acrostone, Egypt).
- Teeth were dried and sectioned with a water-cooled diamond saw in a buccolingual direction through the sealant resulting in three sections for each specimen.
- Each tooth section was examined twice by stereomicroscope (Zeiss Stemi 305 HD Digital Stereo Microscope) at 60X magnification.

- The extent of dye penetration was assessed for all sections according to the following SCORES[10]. Score 0; No dye penetration.
- **Score 1**; Dye penetration restricted to the outer half of the sealant.
- **Score 2**; Dye penetration extended to the inner half sealant.
- **Score 3**; Dye penetration into the underlying fissure.

The section, which was most dye infiltrated, was considered and recorded as the score for the specimen.

3. Evaluation of sealant retention:

- Each specimen was mounted in a special attachment in a Universal Testing Machine (Instron, Comten Industries, USA)
- Shear bond strength was then measured by determining the force required to dislodge the sealant from the enamel surface. Bond strength was calculated in Megapascals (MPa) by

dividing the load at failure (Newtons) by the adhesive surface area of the attachment (mm^2).

Statistical analysis

Numerical data were summarized using mean, standard deviations and were analyzed using the One-Way ANOVA test. Categorical data were expressed as numbers and percentages and were analyzed using the Chi-square test. The statistical values were considered significant at $P< 0.05$.

RESULTS

1. Resin Penetration:

The results showed that the pretreatment of the enamel of pit and fissures with acid-etching alone (group A) “control group” resulted in a **significant** increase in the resin penetration depth when compared to other tested groups **table (1) Fig (2)**.

However, the results showed that the pretreatment of the enamel of pit and fissures with air abrasion or ultrasonic in combination with acid etch (group D and group E) resulted in a **significant** increase in the resin penetration depth when compared with air abrasion or ultrasonic alone (group B and group C).

Moreover, the use of ultrasonic alone (group C) resulted in a **significant** increase in the resin penetration depth when compared with air abrasion alone (group B).

However, the use of ultrasonic in combination with acid etch (group E) resulted in an **insignificant** increase in the resin penetration depth when compared with air abrasion in combination with acid etch (group D).

2. Marginal Adaptation:

Microleakage scores for all tested groups:

The score 0; recorded for acid etching (group A) was present in 9 samples with (75%), **table (2)&(3) Fig (2)** followed by a total number of 8 samples in both air abrasion and ultrasonic in combination with acid etch groups (group D and E) with the percentage of (66.67%), followed by ultrasonic alone (group C) with total samples of 7 and percentage of (58.33%), and air abrasion alone group (group B) with total samples of 6 and percentage of (50%).

However, score 1; recorded a total of 4 samples with a percentage of (33.33%) for air abrasion alone group (group B), followed by a total of 3 samples with a percentage of (25%) for ultrasonic with acid etching group (group E), and finally, a total sample of 2 and percentage of (16.67%) for acid etching, ultrasonic alone, and air abrasion with acid etch groups (group A, C, and D), While, score 2; recorded a total of 2 samples with the percentage of (16.67%) for both of ultrasonic alone and air abrasion with acid etch groups (group C, and D), followed by only 1 sample with the percentage of (8.33%) for the other groups. Finally, score 3; recorded only 1 sample with a percentage of (8.33%) for both air abrasion alone and ultrasonic alone groups (group B, C). while, no other groups recorded a score of 3 among the other tested groups.

3. Retention:

The lower (mean ± SD) values of the resin retention were recorded with air abrasion alone (group B) (64.51±6.02 MPa), **table(4)** followed by ultrasonic alone (group C) (86.37±10.87 MPa), and air abrasion in combination with acid etch (group D) (114.32±5.88 MPa), and ultrasonic in combination with acid etch (group E) (117.14±4.83 MPa) respectively. While, the highest (mean ± SD) value of resin retention was recorded with acid etch alone (group A) (133.03±7.24 MPa).

The results showed that the pretreatment of the enamel of pit and fissures with air abrasion or ultrasonic in combination with acid etch (group D and group E) resulted in a **significant** increase in the resin retention when compared with air abrasion or ultrasonic alone (group B and group C). Moreover, the use of ultrasonic alone (group C) resulted in a **significant** increase in resin retention when compared with air abrasion alone (group B). However, the use of ultrasonic in combination with acid etch (group E) resulted in an **insignificant** increase in the resin retention when compared with air abrasion in combination with acid etch (group D).

TABLE (1) Comparison of percentage of penetration among different groups during the study.

Acid-etching	Air abrasion	Ultrasonic	Air abrasion/AE	Ultrasonic/AE	p-value
87.33±0.93	71.07±1.23	75.99±1.61	81.66±1.60	82.71±1.69	
	P1<0.001*	P2<0.001* P3=0.003*	P4<0.001* P5<0.001* P6<0.001*	P7=0.009* P8<0.001* P9<0.001* P10=0.71690	P<0.001*

*; *The results statistically at P<0.05. P1: AE and Air abrasion, P2: Between AE and Ultrasonic, P3: AE and Air abrasion/AE, P4: AE and Ultrasonic/AE, P5: Air abrasion and Ultrasonic, P6: Air abrasion and Air abrasion/AE, P7: Air abrasion and Ultrasonic/AE, P8: Ultrasonic and Air abrasion/AE, P9: Ultrasonic and Ultrasonic/AE, and P10: Air abrasion/AE and Ultrasonic/AE*

TABLE (2) Comparison between marginal adaptation among different groups during the study.

	Acid-etching	Air abrasion	Ultrasonic	Air abrasion/AE	Ultrasonic/AE	p-value
Success	9 (75%)	6 (50%)	7 (58.33%)	8 (66.67%)	8 (66.67%)	
Failure	3 (25%)	6 (50%)	5 (41.67%)	4 (33.33%)	4 (33.33%)	
		P1= 0.20590	P2=0.38647 P3=0.68204	P4=0.65336 p5=0.40762 P6= 0.6732	P7=0.65336 p8=0.40762 P9= 0.6732 P10= 1.000	P=0.004*

*; *The results statistically at P<0.05. P1: AE and Air abrasion, P2: Between AE and Ultrasonic, P3: AE and Air abrasion/AE, P4: AE and Ultrasonic/AE, P5: Air abrasion and Ultrasonic, P6: Air abrasion and Air abrasion/AE, P7: Air abrasion and Ultrasonic/AE, P8: Ultrasonic and Air abrasion/AE, P9: Ultrasonic and Ultrasonic/AE, and P10: Air abrasion/AE and Ultrasonic/AE*

TABLE (3) Comparison between marginal adaptation among different groups during the study.

Variable	Score 0	Score 1	Score 2	Score 3	Chi-square	p-value
Acid Etch; n (%)	9 (75%)	2 (16.67%)	1 (8.33%)	0 (0.0%)	**5.772**	**0.927 ns**
Air Abrasion; n (%)	6 (50%)	4 (33.33%)	1 (8.33%)	1 (8.33%)		
Ultrasonic; n (%)	7 (58.33%)	2 (16.67%)	2 (16.67%)	1 (8.33%)		
Air abrasion/AE; n (%)	8 (66.67%)	2 (16.67%)	2 (16.67%)	0 (0.0%)		
Ultrasonic/AE; n (%)	8 (66.67%)	3 (25%)	1 (8.33%)	0 (0.0%)		

*; *The results statistically at P<0.05.*

TABLE (4) Comparison between sealant retention among different groups during the study.

Acid-etching	Air abrasion	Ultrasonic	Air abrasion/AE	Ultrasonic/AE	p-value
133.03±7.24	64.51±6.02	86.37±10.87	114.32±5.88	117.14±4.83	
	P1<0.001*	P2<0.001* P3<0.002*	P4=0.001* P5<0.001* P6=0.001*	P7<0.007* P8<0.001* P9<0.001* P10= 0.960	P<0.001*

*; *The results statistically at P<0.05. P1: AE and Air abrasion, P2: Between AE and Ultrasonic, P3: AE and Air abrasion/AE, P4: AE and Ultrasonic/AE, P5: Air abrasion and Ultrasonic, P6: Air abrasion and Air abrasion/AE, P7: Air abrasion and Ultrasonic/AE, P8: Ultrasonic and Air abrasion/AE, P9: Ultrasonic and Ultrasonic/AE, and P10: Air abrasion/AE and Ultrasonic/AE*

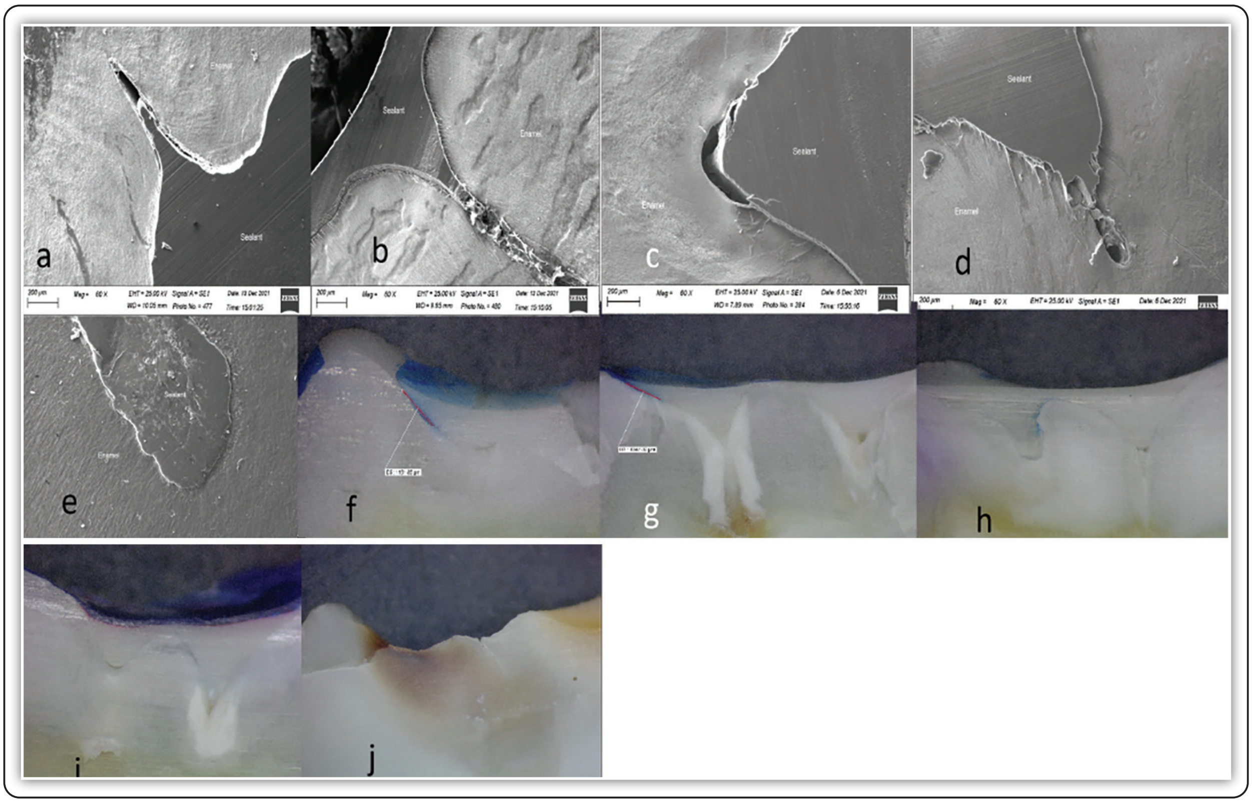

FIG (2) SEM photographs showing resin penetration of an Acid-etching, b, Air abrasion, c, Ultrasonic, d, Air abrasion followed by acid-etching, e, Ultrasonic followed by acid-etching, f, absence of the dye penetration at the sealant-enamel interface of Acid-etching, g, Air abrasion, h, Ultrasonic, i, Air abrasion followed by acid-etching, and j, Ultrasonic followed by acid-etching.

DISCUSSION

The present results showed that the pretreatment of the enamel of pit and fissures with acid etching alone (group A) resulted in a significant increase in the resin penetration depth when compared to other tested groups. Moreover the pretreatment of the enamel of pit and fissures with air abrasion or ultrasonic in combination with acid etch (group D and group E) resulted in a significant increase in the resin penetration depth when compared with air abrasion or ultrasonic alone (group B and group C).

In agreement with our results, Bhushan et al., 2017 [11] assessed and compared the retention of pit and fissure sealants placed using acid etch alone and a combination of air abrasion and acid etch techniques. Combining air abrasion pretreatment with subsequent acid etching did not result in a statistically significant difference in sealant retention compared to acid etching alone. An additional air abrasion pretreatment step can be avoided in pediatric patients when placing sealants and the procedure can be completed faster with better behavior management using acid etching alone.

Also In agreement with our results, Ramesh et al., 2011 [12] visualize the resin tags after enamel conditioning with 37% phosphoric acid or air abrasion. The evaluation showed that enamel conditioning with 37% phosphoric acid produced greater depths of resin penetration than did self-etching primer or air abrasion. However, the results of the current study showed that the use of air abrasion and ultrasonic abrasion in combination with acid etching resulted in an improvement in resin penetration. These results could be attributed to the removal of organic plug material from deep pits and fissures by the action of acid etching, thereby allowing deeper penetration of both etchant and sealant material [13].

In the present study, the fissures pretreated with air abrasion or ultrasonic followed by acid etching, and the fissures pretreated with acid etching alone exhibited less microleakage than did those in the other experimental groups. This finding was consistent with the results of Lupi-Pégurier et al., [14] microleakage of sealants prepared with air abrasion alone displayed significantly greater microleakage (80%) ($p < 0.0001$) than the ones placed after prophylaxis and etching (13.33%), etching (20%), or air abrasion and etching (22.2%). Air-abrasion treatment does not eliminate the need for etching the enamel surface before applying the sealant. This could be attributed to the that air abrasion treatment resulted in a smoother, and less retentive surface, resulting in high microleakage, while acid etching of the enamel causes a selective dissolution of the inorganic component of the enamel matrix creating a more retentive surface and hence resulting in less microleakage [2]. A possible cause for the high microleakage scores in the air abrasion group might be the incomplete penetration of the sealant into the fissure as a result of the residual sodium hydrogen carbonate particles that might remain in the fissure after treatment. This finding is in agreement with the findings of Davis et al [15].

The results showed that the pretreatment of the enamel of pit and fissures with acid-etching alone (group A) resulted in a significant increase in the resin retention when compared to other tested groups. Moreover , the results showed that the pretreatment of the enamel of pit and fissures with air abrasion or ultrasonic in combination with acid etch (group D and group E) resulted in a significant increase in the resin retention when compared with air abrasion or ultrasonic alone (group B and group C). Also , the use of ultrasonic alone (group C) resulted in a significant increase in resin retention when compared with air abrasion alone (group B). However, the use of ultrasonic in combination with acid etch (group E) resulted in an insignificant increase in the resin retention when compared with air abrasion in combination with acid etch (group D).

The significantly higher results of acid etching could be attributed to the fact that the alteration in the enamel achieved by air abrasion or ultrasonic, microscopically, is not the same as for acid etch, and may not have optimal mechanical retention properties [2]. As the longevity of fissure sealants placed is related to the presence of sufficient bond strength between the tooth surface and the material, and to the retention of the sealant material [16].

On the other hand, Kanellis et al. 2000 [17], reported that the use of these approaches (air abrasion and ultrasonic) alone does not produce higher bond strengths in vitro. Sapanpuneet [18] assessed the retention of pit and fissure sealants placed using acid etch alone and a combination of air abrasion and acid etch techniques. There was no significant difference in retention of pit and fissure sealants in either technique air abrasion treatment resulted in an irreversible removal of both the organic and inorganic components of the enamel matrix, producing a smoother, and hence a less retentive surface, while acid etching of the enamel causes a selective dissolution of just the inorganic component of the enamel matrix creating a more retentive surface. Bendinskaite et al., 2010 [19] evaluated the status of sealed occlusal surfaces using the air-abrasion and sealants using the acid etching method. The differences between the two methods: air-abrasion and acid etching in terms of sealant retention rate and caries development appeared to be statistically insignificant air abrasion may force alumina particles and plaque deeper into pits and fissures. This may impede acid and sealant penetration and prevent adequate bonding.

CONCLUSION

According to the findings of the present study, the following conclusion can be drawn:

1. Conventional acid etching remains the most effective and simplest technique for sealants' success.
2. The use of air abrasion or ultrasonic alone was inadequate for etching enamel before sealant application.
3. The use of air abrasion followed by acid etching can be recommended to maintain an adequate seal.

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