Effects of Fissure Cleaning Methods, Drying Agents, and Fissure Morphology on Microleakage and Penetration Ability of Sealants In Vitro

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Abstract

Purpose: The purpose of this in vitro study was to determine the effect of different cleaning methods and the use of post-etching drying agents, as well as the influence of fissure types on the microleakage and penetration ability of fissure sealants.
Methods: Ninety extracted human molar teeth were randomly divided into 9 groups of 10 each. The treatment groups were defined by the combination of different cleaning methods (traditional pumice prophylaxis, air abrasion with Prophyflex, and air abrasion with Airflow handy) and different drying agents (no drying agent, alcohol 99%, and acetone 99.5%). The teeth were thermocycled (5,000 cycles at 5°C-55°C) and were then immersed in a solution of 5% methylene blue for 24 hours. Microleakage, penetration ability, and fissure types were examined after sectioning. Multiple regression analyses were used for statistical analysis.
Results: No significant difference was found in microleakage and the penetration ability between the use of different cleaning methods and sealant placement with or without drying agents ($P>.05$). Y2 fissure type exhibited significantly higher unfilled areas than other fissure types ($P<.001$).
Conclusions: Neither air abrasion with acid etching nor the use of post-etching drying agents decreased microleakage and improved the penetration ability of sealants significantly compared to the conventional sealant application. Fissure type was significantly related to the penetration ability of sealants. (*Pediatr Dent*. 2003;25:527-533)

KEYWORDS: PIT AND FISSURE SEALANT, AIR ABRASION, DRYING AGENT, FISSURE TYPE

Received October 18, 2002   Revision Accepted April 25, 2003

The caries-preventive effectiveness of pit and fissure sealants has been well documented for the past 20 years.[1] Long-term retention of the sealants and their tight micromechanical adhesion to enamel surfaces are essential for their success. Conditioning surface enamel with phosphoric acid is the standard method for preparing the enamel surface prior to sealant placement.[2] A significant advantage has been demonstrated in having a clean, debris-free surface before etching.

Various pretreatment methods have been investigated with the intent to enhance the effectiveness of etching the enamel surface. However, there is no consensus relative to the best method of cleaning pits and fissures prior to applying etchant and sealant. The use of pumice slurry with rotary instruments in a low-speed handpiece to clean the tooth is the method most widely accepted by general dental practitioners.[2,3]

Nevertheless, pumice prophylaxis does not completely and consistently remove the pellicle and debris especially in the deep part of fissures.[4] Even after etching and rinsing, debris, may remain in the pits and fissures, preventing enamel conditioning and decreasing resin penetration.[5,6] Recently, air abrasion has been suggested as a better pretreatment method to mechanically roughen enamel in a conservative and time-efficient manner and remove residual organic material from the fissures. It can remove debris, excavate incipient decay, and eliminate the need for acid application and rinsing steps.[7-9]

However, other studies comparing bond strength have shown that air abrasion alone is not sufficient for promoting a high bond strength of a sealant to enamel.[10-12] A combination of air abrasion and phosphoric acid etching yielded better results in decreasing microleakage and increasing bond strength than acid etch conditioning without air abrasion.[12] In contrast, other authors reported that air abrasion followed by acid etching did not produce significantly less microleakage than traditional pumice prophylaxis with acid etching technique.[13]

Table 1. The Treatment Groups

Group	Cleaning method	Etching	Drying agent
1	Brushing with nonfluoridated paste	Yes	No drying agent
2	Brushing with nonfluoridated paste	Yes	Primadry (alcohol 99%)
3	Brushing with nonfluoridated paste	Yes	Acetone 99.5%
4	Air abrasion with Prophyflex	Yes	No drying agent
5	Air abrasion with Prophyflex	Yes	Primadry (alcohol 99%)
6	Air abrasion with Prophyflex	Yes	Acetone 99.5 %
7	Air abrasion with Airflow handy	Yes	No drying agent
8	Air abrasion with Airflow handy	Yes	Primadry (alcohol 99%)
9	Air abrasion with Airflow handy	Yes	Acetone 99.5%

Complete penetration of resin into the complex fissure system is hard to attain, especially into the deep and narrow fissures[3,14] due to the phenomenon of "close-end capillaries" or "isolated capillaries."[15] Recently, the use of post-etching drying agent (Primadry, Ultradent, USA) has been proposed as an additional step of sealant procedures to reduce humidity to a minimum by volatilizing the residue moisture content of fissures. An in vitro study indicated that the use of a drying agent could enhance the penetration ability of hydrophobic sealants into the dried etched pattern by increasing the surface energy of etched surfaces.[16] Controversially, a study by Rix et al, reported no statistical differences between the retention rates of fissure sealants with and without the use of a drying agent.[17]

The susceptibility of occlusal surfaces to caries has often been related to the morphology of pits and fissures on these surfaces.[18] It was suggested that the internal morphology of the groove-fossa system influenced the conditions for bacterial growth and this determined the location for caries progression.[19] However, little information has been reported on the effect of fissure morphology on the sealant microleakage or the penetration ability of sealant materials. This raises the question whether deep pits and fissures are related to the successful placement and retention of fissure sealants.

Therefore, the aim of this in vitro study was to determine the effect of cleaning methods, the use of postetching drying agents and the influence of fissure types on the microleakage and penetration ability of fissure sealants.

Methods

Ninety extracted human third molar teeth—free of caries, fluorosis, fissure sealants and restorations, which had previously been stored in Chloramine 1%—were selected by visual inspection and using Diagnodent (KaVo, Biberach, Germany).[20] After that, the teeth were randomly assigned to 9 treatment groups with 10 teeth each. All procedures were performed in a humidity chamber (relative humidity 90%±2%) under room temperature (25°C±2°C).

The steps used for all procedures were standardized as follows (Table 1):

1. Cleaning method:
    a. Groups 1 to 3—using a bristle brush with nonfluoridated paste (Pell-ex, Hawe-Neos Dental, Bioggio, Switzerland) for 15 seconds.
    b. Groups 4 to 6—air abrasion with Prophyflex using sodium bicarbonate particles (Kavo, Biberach, Germany) for 15 seconds according to the manufacturer's instructions.
    c. Groups 7 to 9—air abrasion with Airflow handy using sodium hydrogen carbonate particles (EMS, Nyon, Switzerland) for 15 seconds according to the manufacturer's instructions.
2. Rinse for 20 seconds with an airwater syringe (all groups).
3. Dry with oil-free compressed air for 15 seconds (all groups).
4. Etch with a 35% phosphoric acid gel (Ultra Etch, Ultradent Products Inc., USA) for 60 seconds. During the etching process, the etchants were gently moved on the occlusal surfaces by using a soft microbrush to enhance the penetration ability of the etchant into the fissure systems[3] (all groups).
5. Rinse for 30 seconds with an airwater syringe (all groups).
6. Dry with oil-free compressed air for 15 seconds (all groups).
7. The use of post-etching drying agents:
    a. Groups 1, 4, 7—no drying agent was used.
    b. Groups 2, 5, 8—alcohol 99% (Primadry, Ultradent Products Inc, USA) was applied on the etched occlusal surfaces for 10 seconds. After that, the fissures were dried off with oil-free compressed air for 10 seconds.
    c. Groups 3, 6, 9—acetone 99.5% (Merck KGaA, Damstadt, Germany) was applied on the etched occlusal surfaces for 10 seconds. After that, the fissures were dried off with oil-free compressed air for 10 seconds.

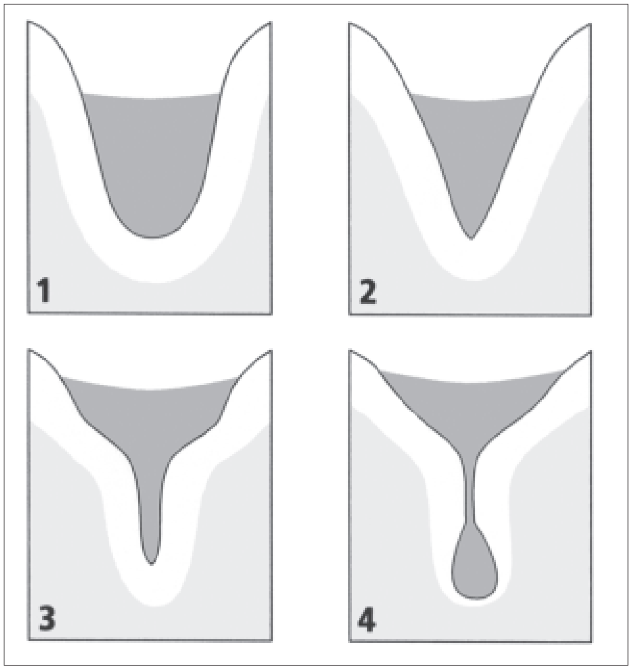

Figure 1. Diagrammatic depiction of the micromorphological types of fissure system.
1=U-type; 2=V-type; 3=Y1-type; 4=Y2-type.

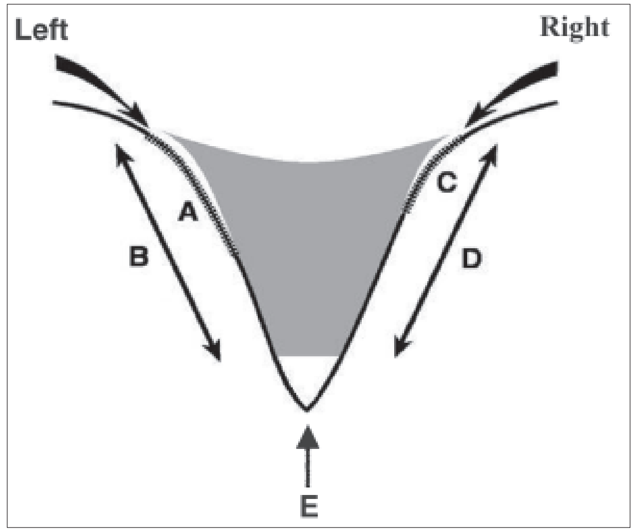

Figure 2. Scoring system employed for the evaluation of microleakage and penetration ability.
A+C=length of dye penetration; B+D=length of sealant-tooth interface;
$\frac{A+C}{B+D}$=proportion of microleakage; E=unfilled area (mm^2).

8. Sealant application: The sealing material (Concise, unfilled white sealant, 3M Espe Dental Products, St. Paul, Minn) was applied using an explorer without loading on the occlusal surfaces with a sealant penetration time of 20 seconds before applying the light activation. Then, the sealants were cured using an Astralis 7 (750 mW/cm,[2] Vivadent, Schaan, Liechtenstein) for 40 seconds. Care was taken not to place too much sealant material on each occlusal surface.[3]

Thermocycling and dye penetration

Following sealant placement, the teeth were thermocycled in water for 5,000 cycles between 5°C± 2°C and 55°C± 2°C with a dwell time of 30 seconds. The surfaces of the teeth were then coated with melted utility wax, leaving the sealant and approximately 1.5 mm uncovered around the sealant. The coated teeth were immersed in 5% methylene blue for 24 hours to allow dye penetration into possible gaps between the tooth substance and the sealant.

Microscopic examination

For further examination, the coatings were stripped off and the teeth were rinsed thoroughly with tap water and embedded in self-curing resin to prevent the chipping of material. The teeth were then sectioned with 3 parallel cuts in the bucco-lingual direction with a low-speed saw (Isomet, Buehler, USA), yielding 3 surfaces per tooth for analysis. Fissure type, microleakage, and penetration ability were evaluated using a light microscope, at a magnification of ×25 (Wild, Leitz Ltd, Heerbrugg, Switzerland), equipped with a video camera linked to the computer. The examiner was blind to the groups. Optimas software (BioScan Inc., Washington, USA) was used to measure the length of dye penetration, the enamel-sealant interface (mm^2), and the unfilled area of fissures (mm^2).

Examination of fissure type

The micromorphological types of the fissure system were classified as follows[21] (Figure 1): (1) U-type; (2) V-type; (3) Y1- type; (4) Y2-type.

Examination of microleakage and penetration ability

The dye penetration value per surface was evaluated as shown in Figure 2. Three parameters were evaluated following Zyskind et al.[10] Lack of vertical sealant adaptation was identified by the presence of gaps between the sealant and the fissure wall or by the absence of sealant penetration into the fissures. The penetration ability was expressed as the unfilled area (mm^2) of fissures (Figure 2). The value of the measurement in each surface and the mean value per tooth were adopted as the representative values.

Statistical analyses

The data were analyzed by general descriptive and multivariable methods using the general linear model procedure of the SYSTAT software for data analysis (SYSTAT, Inc., Evanston, Ill, USA). At surface level analysis, the microleakage values and unfilled areas (mm^2) were subjected to multiple regression analyses to test whether the independent variables (cleaning methods, drying agents, and fissure types) influenced the performance of fissure sealants (microleakage and the penetration ability). At tooth level analysis, the data were subjected to the same test used

Table 2. Microleakage and Unfilled Area (Mean±SD) for Different Treatment Groups*

Group section(N)	Cleaning method	Drying agent	Microleakage (mean±SD)	Unfilled area (mean±SD)
1	Brushing	No	0.231±0.306	0.036±0.082
2	Brushing	Alcohol 99%	0.216±0.278	0.012±0.029
3	Brushing	Acetone 99.5%	0.202±0.303	0.04±0.114
4	Prophyflex	No	0.277±0.343	0.029±0.057
5	Prophyflex	Alcohol 99%	0.174±0.246	0.013±0.04
6	Prophyflex	Acetone 99.5%	0.141±0.279	0.01±0.024
7	Airflow handy	No	0.133±0.256	0.019±0.046
8	Airflow handy	Alcohol 99%	0.154±0.32	0.011±0.019
9	Airflow handy	Acetone 99.5%	0.147±0.166	0.016±0.04

*No significant difference was found between the treatment groups (P>.05).

at surfacelevel analysis. However, the type of fissures was not included as an independent factor at tooth level analysis. Differences between subgroups were checked for significance using Tukey's analysis. The level of significance was set at P<.05.

Intraexamination reliability

Nine teeth (10% of all teeth) were randomly selected and re-examined by the same examiner under the same conditions and using the same equipment. The intraexamination reliability was tested using Cohen's unweighted kappa statistic (fissure type) and Spearman rank correlation coefficients (microleakage and penetration ability).

Results

A total of 254 sectioned surfaces were examined for microleakage, unfilled area, and fissure type (16 missing values from 270 sections). The percentage of the samples without microleakage in each group was as follows: Group 1=41%, Group 2=38%, Group 3=52%, Group 4=41%, Group 5=56%, Group 6=77%, Group 7=32%, Group 8=64%, Group 9=29%. The mean (±SD) microleakage of all groups are presented in Table 2. The use of air abrasion in combination with drying agents (groups 5, 6, 8, and 9) showed slightly lower microleakage than the control group (Group 1), however, no significant difference existed between these groups (P>.05). When tested by Tukey's analysis, there were no statistically significant differences in microleakage between different cleaning methods and drying agents. Although the Y1 fissure type produced lower sealant microleakage than other fissure types, no significant differences were found in microleakage between different fissure types (P=.054; Table 3). As can be seen in Table 4, no variables analyzed at the section and tooth level were significantly related to sealant microleakage.

The distribution of unfilled areas (mm^2) for all groups is shown in Table 2. The use of air abrasion and/or the use of drying agents (groups 4-9) exhibited smaller unfilled areas than the control group (Group 1) but the differences were not statistically significant (P>.05). No significant difference was found in unfilled areas between sealant placement with or without drying agents when analyzed at the surface and tooth level. On the other hand, the only variable significantly related to the penetration ability was the type of fissures (P<.001; Table 4). As can be seen in Table 3, Y2 fissure type exhibited significantly higher unfilled areas than other fissure types (P<.001).

Results from the duplicate examination showed that the intraexamination reliability on fissure type as assessed by Cohen's unweighted kappa statistic was good (κ=0.8) and the intraexamination reliability of the microleakage and penetration ability (unfilled area) as tested by Spearman rank correlation coefficients were 0.86 and 0.91, respectively which indicated good reproducibility.

Discussion

The retention of sealants is a mechanical process established by resin tag formation into the porosity created in the etched enamel.[22] Even under proper conditions of application, sealant failures can be expected at 5% to 10 % per year.[23] A variety of fissure preparation methods have been investigated in an attempt to successfully inhibit microleakage and improve sealant retention. However, published findings have revealed conflicting results, especially the need of additional pretreatment methods prior to sealant placement. It was suggested that conventional pumice prophylaxis left fissures filled with residue and pumice, supporting previous studies by Brown et al, and Burrow et al.[5,6] In the past several years, the use of air abrasion has been advocated to clean plaque and extrinsic stain from fissures, as it consistently removed residual integument in pits and fissures.[7] In the present study, no significant differences in microleakage were found between conventional pumice prophylaxis and 2 air abrasion systems in combination with acid etching. A recent study by Blackwood et al yielded similar results.[13] They demonstrated no significant difference in microleakage when comparing cleaning with pumice to air abrasion. In contrast, prophylaxis with a rubber cup or pointed bristle brush with or without pumice showed the lesser penetration of resin into the etched enamel compared to modified methods tested for occlusal fissure cleansing.[4] The penetration ability and microleakage of the sealants with air abrasion alone (without acid etching) were not tested in this study because it was previously shown that the roughed surface created by air abrasion without acid etching lacks the seal obtained with acid etching alone.[9]

Table 3. Microleakage and Unfilled Area (Mean±SD) for Different Fissure Types

Fissure type	No. of sections (%)	Microleakage (mean±SD)	Unfilled area* (mean±SD)
U type	80 (32%)	0.227±0.327	0.008±0.062
V type	53 (21%)	0.202±0.307	0.001±0.004
Y1 type	79 (31%)	0.118±0.194	0.024±0.048
Y2 type	42 (17%)	0.222±0.283	0.064±0.081

*Groups connected by a line are different at the 5% significant level.

The results of the authors' study suggest that neither air abrasion followed by acid etching nor the use of drying agents produced significantly less sealant microleakage than the traditional pumice prophylaxis. Likewise, it made no difference what type of fissure preparation is used as long as it was followed by acid etching prior to sealing.[13] Therefore, the need to routinely air abrade prior to etching on the sound enamel is not supported by the present study as it requires a special device, and probably additional time to clean the working area from the substantial dust powder.

Although the procedures were performed in a humidity chamber, this was an in vitro study under relatively ideal conditions. Within the limits of this study, such factors as variation between teeth, quality of pellicle or debris (ie, thickness, adherence) may cause variation of the results obtained in vivo. For instance, the amount of bacterial plaque in vitro would not be similar to in vivo conditions. Furthermore, this study was mostly conducted on extracted third permanent molars which usually have different groove-fossa systems compared to the first and second molars. As a result, the interaction of the treatments used in the authors' study when conducted in vivo may differ.

In the present study, each tooth was sectioned with 3 parallel cuts, yielding 4 sections (thickness=approximately 1.5-2 mm). Due to the fact that the thickness of a diamond blade is rather thin (0.3 mm), 2 adjacent surfaces (mirror images) are dependent on each other. If both of the mirror images in 1 cutting had been used for analysis, double sample sizes would have been obtained, thus artificially inflating the power of the experiment. Therefore, only 1 surface in each cutting was evaluated, thus obtaining 3 independent surfaces per tooth for analysis.

Much of the research about sealant success has addressed retention, bond strength and microleakage, but little information has been reported on the relationship between the penetration ability of sealants (unfilled areas) and different materials and methods used. In the present study, no significant difference in unfilled areas between pumice prophylaxis and air abrasion was found. In addition, sealant application with or without the post-etching drying agents demonstrated similar results. In other words, no significant correlation could be established between the modified methods and the penetration ability of sealant.

In contrast, Kersten et al, demonstrated that the application of acetone prior to sealant placement, in combination with other modified techniques, could improve the penetration depth of resin into the fissures and into the conditioned enamel.[16] The difference in findings may be due to the use of different methods, parameters, and variables. First, to simulate oral condition, the procedures in the present study were performed in a humidity chamber (relative humidity 90%±2%). For this reason, it might affect the potential of drying agents to volatilize the residue moisture compared to the use of drying agent in ambient room conditions. Secondly, the authors' penetration ability was expressed as the unfilled area (mm^2), whereas the results by Kersten et al, were obtained from the percentage of penetration depth. Lastly, the fissure morphology, found to be the greatest influence on the penetration ability in the authors' study, was not evaluated in the study by Kersten et al. These controversial results could be explained by the effect of confounding.[24] Confounding is a mixing of the effects on an outcome of 2 or more independent variables, at least 1 of which is not captured (controlled for) by the study design. Therefore, the effect of the use of post-etching drying agents on the penetration ability might be not found when the type of fissures was introduced to the analysis.

Therefore, it could be concluded that the use of post-etching drying agents was not more effective than the sealant application without drying agents at enhancing the penetration of a resin into the fissure systems. In fact, saliva contamination of etched enamel is the most frequently cited reason for sealant failure. Though not contraindicated, the additional step of a drying agent before sealant is not necessary, especially with young children due to the increased time of tooth isolation with the equivalent results. In clinical practice, the critical steps in sealant application might be tooth

Table 4. Results of Multiple Regression Analyses at the Surface and Tooth level Analysis

Dependent factor	The level of analysis	N	*P* value			R^2
			Independent factors			
			Cleaning	drying	fissure type	
Microleakage	Surface level	254	.183	.501	.054	0.047
	Tooth level	90	.202	.563	-	0.049
Penetration ability	Surface level	254	.121	.307	<.001*	0.152
	Tooth level	90	.242	.222	-	0.065

*Indicates a significant interaction (P<.05).

isolation, eruption status of the tooth, patient cooperation, and routine periodic evaluation.

As stated previously, deep fissures did not produce statistically higher microleakage than shallow fissures. Statistical analysis revealed no significant difference in sealant microleakage between different fissure types. Regarding the relationship between leakage and penetration ability, no significant correlation was found between the extent of leakage and the occurrence of unfilled areas at the sealant to tooth interfaces. Therefore, deep and narrow fissures, thought to be more susceptible to caries attack, did not influence the marginal seal at the sealant to enamel interface.

On the other hand, the fissure types were significantly associated with the penetration ability. Penetration of resin sealant to the base of the fissure occurred more frequently in the shallow fissures compared with the deep fissures. This finding coincided with observations reported in previous investigation that the penetration ability of sealants was affected by fissure depth and complexity.[25] Similarly, an in vitro study by Sutalo et al, demonstrated that the penetration ability of the sealants is in relation to the width of the fissure entrance and depth.[21]

Viable microorganisms were primarily observed at the entrance of the deep fissures, while in deeper part the microorganisms were less viable or dead due to the diffusion barrier.[19] If sealant retention is gained from good adaptation of sealant to the enamel, routine removal of sound fissures by enameloplasty to achieve complete penetration of sealants to the bottom of fissures might not be an important step in sealant success. However, to make the investigation of the penetration ability more meaningful, a link between the enhancement of the penetration of resin into deep grooves and their clinical performance must be established.

Conclusions

1. The use of air abrasion in combination with acid etching did not significantly decrease sealant microleakage or improve the penetration ability compared to the traditional pumice prophylaxis with acid etching (P>.05).
2. Compared to conventional sealant application without any drying agents, the additional step of drying agents (either alcohol or acetone) did not produce significantly less sealant microleakage and unfilled areas (P>.05).
3. Fissure type was significantly related to the penetration ability of sealants (P<.05).

Acknowledgements

Dr. Duangthip was supported by a grant from Thammasat University, Thailand.

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Abstract of the Scientific Literature

Root Fractures in Permanent Incisors

Root fractures in permanent incisors account for 0.5-0.7% of all dental injuries. The purpose of this study was to determine the type of healing which occurred in root-fractured permanent incisor teeth in children and whether gender, age, stage of root development, location of the fracture, or treatment affected the outcome. The clinical records of 34 children with root-fractured incisor teeth who were followed up for a minimum of 3 years were examined in this retrospective study. Root development was incomplete in 27 of the teeth and complete in 7 teeth. Twenty-five root fractures occurred in the apical third, 8 in the middle third, and 1 in the coronal third of the root. Results showed that the most common type of healing was by connective tissue followed by calcified tissue. The only factor related to healing was the stage of root development (ie, whether the apex was open or closed). The majority of root-fractured teeth (80%) in this study showed good healing. Those teeth that showed poor healing still had a good prognosis, as the coronal fragment could be endodontically treated.

Comments: It must be noted that the sample size for this study was very small and the follow-up period was 3 years. This study did not find any relationship between healing and location of the fracture, which might be due to the small sample size. **HA**

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