[UCI]I804:24011-200000232679

2006년 2월
박사학위 논문

Effect of dye-guidance brushing etching technique on the performance of pit and fissure sealant

조 선 대 학 교 대 학 원
치 의 학 과
Phan Ai Hung

Effect of dye-guidance brushing etching technique on the performance of pit and fissure sealant

2006년 2 월 일

조 선 대 학 교 대 학 원
치 의 학 과
Phan Ai Hung

Effect of dye-guidance brushing etching technique on the performance of pit and fissure sealant

지도교수 이 상 호

이 논문을 치의학 박사학위 논문으로 제출함

2005년 10월 일

조 선 대 학 교 대 학 원
치 의 학 과
Phan Ai Hung

Phan Ai Hung의 박사학위 논문을 인준함

위원장	원광대학교	교수	이 광 희	인
위원	연세대학교	교수	최 병 재	인
위원	조선대학교	교수	이 상 호	인
위원	조선대학교	교수	김 흥 중	인
위원	조선대학교	교수	이 창 섭	인

2005년 12월 일

조 선 대 학 교 대 학 원

Effect of dye-guidance brushing etching technique on the performance of pit and fissure sealant

2006년 2월 일

조선대학교 대학원

치 의 학 과

Phan Ai Hung

Effect of dye-guidance brushing etching technique on the performance of pit and fissure sealant

지도교수 이 상 호

이 논문을 치의학 박사학위 논문으로 제출함

2005년 10월 일

조선대학교 대학원

치 의 학 과

Phan Ai Hung

Phan Ai Hung의 박사학위 논문을 인준함

위원장　원광대학교 교수　이 광 희　인

위　원　연세대학교 교수　최 병 재　인

위　원　조선대학교 교수　이 상 호　인

위　원　조선대학교 교수　김 흥 중　인

위　원　조선대학교 교수　이 창 섭　인

2005년　12월　일

조선대학교 대학원

Contents

List of Tables

List of Figures

Dye-guidance와 brushing을 통한 산부식 방법이 치면열구전색술의 효과에 미치는 영향

판 아이홍, D.D.S., M.S.D.
지도교수 : 이상호, D.D.S., M.S.D., Ph.D.
조선대학교 대학원 치의학과

본 연구는 치면열구전색술시 치과용 염료를 이용하여 초기 우식병소의유무와 유기물의 잔재를 확인하고 산부식 액의 도포시 brushing 방법을 이용하여 침투를 깊이 유도하므 로써 열구내법랑질의 산부식 상태를 유지력을 얻기 위한 최상의 상태로 만들고자 하는 목적으로 자연치를 대상으로 여러가지 치면세마법과 비교하여 다음과 같은 결과를 얻었다.

1. 실험군은 기존의 치면세마법보다 변연누출이 적었으나 enameloplasty를 시행한 군과는 차이가 없었다.
2. 법랑질 산부식 형태의 질이 향상되었다.
3. 실험군은 다른 군에 비해 미세결합강도가 높았다.
4. 구강내 환경적 조건이 치면열구전색재의 성공실패를 좌우한다.

이상의 결과를 종합하면 치면열구전색술시 치과용 염료와 산부식 액의 도포시 brushing 방법을 이용하여 침투를 깊이 유도하므로써 수복물의 성공적인 도포를 제공할 수 있다고 사료된다.

I. Introduction

Tooth surfaces with pits and fissures are particularly vulnerable to caries development. This can be explained by the morphological complexity of these surfaces, which favours plaque accumulation to the extent that the enamel does not receive the same level of caries protection from fluoride as does smooth surface enamel. The plaque accumulation and caries susceptibility are greatest during the eruption of the molars, and caries susceptibility individuals are therefore vulnerable to early initiation and fast progression of caries in these sites.

The need for surface cleaning and the method of cleaning pits and fissures prior to sealant placement is controversial. One textbook[1] suggested careful removal of plaque and pellicle by the use of pumice or air-polishing instruments in order to obtain optimal acid-etch pattern of the enamel. But Harris[2] maintained that the effect of acid etching alone is sufficient for surface provided obvious soft material has been removed. The literature was extensive on the efficiency of different cleaning procedures on bonding[3], including the use of rotating burs in order to remove superficial enamel and open the fissure to have the resin penetrate into it. However, although cleaning the fissures with a bur has given superior retention in some studies[4,5] there is evidence in other studies that it provides no additional benefit. Therefore, the search continues for the most effective enamel surface preparation to enhance sealant integrity.

An other important issue in sealant placement is isolation. Adequate isolation is the most critical aspect of sealant filling. If the enamel porosity created by the etching procedure is filled by any kind of liquid, the formation of resin tags in the enamel is blocked or reduced, and the resin is poorly retained. The isolation procedure may frequently be extremely challenging, particularly in the partially erupted teeth or in those children with poor cooperation.

The objective of this study was to examine the hypotheses that the conventional etching method in pits and fissures placement could be improved and enhanced by vigorous motion etchant agent using in vitro experiments. The expectation of this study was to find a simple procedure which is suitable for children, but effective than pumicing conventional etching technique. An additional purpose was to examine the influence of environment on the performance of sealant.

The null hypothesis that were tested are:

- the vigorous brushing does not result in performance of sealant.
- the environment (mouth breathing) does not influence the retention of sealing material.

Ⅱ. Materials and methods

Effect of dye-guidance brushing etching technique on the microleakage of sealant (Fig. 1).

Seventy extracted sound human permanent third molars and premolars, which had been stored in saline, were used in this study. The teeth were randomly divided into 3 groups. All fissures were gently cleaned with a toothbrush under tap water to remove gross debris. Occlusal fissures were cleaned with a rotary brush and fluoride-free prophylaxis paste and rinsed with tap water, except testing group (no prophylaxis). The pit and fissure was treated with different techniques corresponding to the different experimental groups:

- Conventional group: Occlusal fissures were conventionally etched with 35% phosphoric acid gel (Ultra Etch®, Ultradent Products Inc., USA) for 30 s and thoroughtly rinsed and dried.

- Enameloplasty group: Occlusal fissures were opened with a fine grit, thin flame-shaped diamond bur (Dia-Burs TC-11F, Mani Inc., Japan) with water-spray coolant, etched with phosphoric acid gel for 30 s.

- Modified etching group: The plaque detector (Red-Cote, Butler Gum, John O. Butler Company Chicago, IL 60630 USA) was applied on occlusal surface for 5 s, dried, rinsed to remove excessive dye, and dried again. Etched with phosphoric acid gel for 30 s. During the first 15 s of total etched time, the etchant was vigorous brushed on the stained pit and fissure surfaces by using a microbrush (Inspiral®Brush tip, UltraDent, USA), rinsed and dried. The brush length in the microbrush were standardized at 1.2 mm.

The sealant (Clinpro™, 3M, USA) was carefully applied to all conditioned fissures (border-filled) and cured with light curing unit (Ultra Lite 180A Plasma, Xenon Arc Lamp, Rolence Enterprice Inc.) for 6 s.

After storage in distilled water at room condition, the restored teeth were subjected to artificial aging by thermocycling 600 cyles at 5ºC and 55ºC with a dwell time of 30 s at each temperature. The specimens were then immersed in 1% methylene blue dye at room condition for 24 h. After thermocycling, each specimen was mounted with an acrylic block and sectioned with 3-5 parallel cuts in 4 to 5 slices of 1-1.25 mm thickness per tooth, depending on tooth size, in bucco-lingual direction with a low-speed water-cooler diamond saw (Isomet, Buehler, Lake Bluff, IL, USA). Four teeth in which caries were detected during sectionning were discarded.

All the sections were coded anonymously and randomly mixed before being examined, scored under a stereo microscope by trained examiner at different magnifications from x20 to x40 according to the following scale:

- 0: no dye penetration.
- 1: dye penetration restricted to the outer half of the fissure.
- 2: dye penetration restricted to the inner half of the fissure.

- 3: dye penetration restricted into underlying of the fissure.

If there were multiple fissures on the same sectioned side, the worst score was recorded. In addition, penetration ability, fissure type and voids were examined. The penetration scores were (the sealant penetrated into the fissure or into the space widen by bur):

- 1: sealant penetration into 1/3 of the fissure.
- 2: sealant penetration into 2/3 of the fissure.
- 3: sealant penetration all of the fissure.

Non parametric Kruskal-Wallis and Mann-Whitney tests were used for statistical analysis.

Effect of modified etching procedure compared with conventional etching method which was directly applied on the surface of fissure.

A group of 9 healthy extracted upper premolars were used. Marking the entrance of the pit and fissures by scratching on the enamel approximate 1-5 μm in depth with round diamond bur.

The roots were cut off with a diamond disc under running water, just below the cemento-enamel junction, and then a slot was cut from the cemento-enamel junction towards the fissure. The crown was fractured through the fissure. Only five paired samples with the actual deep fissure were selected. The fissure surface was washed under running water to remove gross or loosen remnants. On the haft of each paired surface, the etching procedure was done directly. All paired specimens were prepared and examined by a SEM (XL30 Series, Philips Electronic Instruments Inc., USA). The whole of specimens was first examined under lowest magnification (x30). The etched pattern was examined at the entrance (under scratched line) and at the bottom of the fissure. Four sites was randomly chosen and captured at x1000 magnification in each area: two lateral sites and two central ones.

The image collected from the SEM examination was printed by ASCE 7 software. The surface alterations of the specimens were quantified by calculating the percentage of porosity present on each of the image using a transparent grid overlay. The grid overlay provided 40 equal cells and was placed over the printed image and the number of cells containing the defined surface alterations was recorded and calculated (40 cells = 100%).

The surface alterations (etched pattern) were defined in following way (Fig. 2):

1: (Well defined) outline of individual enamel prisms clearly visible (type 1 and type 2 etched pattern).

2: (Poorly defined) spectrum of intermediate surface irregularities (pitted etched pattern).

3: (Unetched) relatively smooth enamel surface.

4: (Uncleaned) debris or remnant remained.

A paired t-test was applied to the amassed data.

Effect of modified etching technique on the penetration of sealant into pit and fissure region in replica study.

The material comprised 13 erupted premolars. The roots of each individual tooth were cut off and scratched mark of the order of 1-5 μm in depth was made with round diamond bur on the entrance of pits and fissures.

The occlusal surface was divided into two parts by cutting line (by a diamond disc) in bucco-lingual direction and perpendicular with the occlusal surface. A half (of occlusal surface of the tooth) was received one of two following procedures: the conventional etching technique and the rest with the modified technique previous described. Sealant applying was performed.

The specimens were immersed in concentrated hydrochloride acid until the tooth substance was completely removed (48 h). Subsequently, the obtained replica was then cleaned in 6% sodium hypochloride for 15 min. Rinsed and dried. Two paired dimensions of pit and fissure in each replica were done for each specimen. The longest (depth) of sealant replica along the vertical full line (from the marked entrance to the bottom, perpendicular with occlusal surface) was measured under stereoscope at 2 points of each half: 1 point was located at the 0.5 mm next to the cutting line, the other was at proximal pit. Two damaged specimens were excluded.

A paired t-test was applied to the amassed data.

Effect of modified etching technique on the bond strength of sealant on the etched enamel compared with conventional etching method (Fig. 3).

Dental pieces (3 mm x 6mm) which have flat enamel surface were obtained. They were embedded in plastic tubes of 22 mm in diameter and 30 mm in height with the enamel surfaces parallel to the border of the tube. Slice of 3 mm in thickness was cut perpendicular using a slowly rotating diamond blade under water lavage along the long axis of the resin embedded specimen.

The half of each surface was received one of two etching procedures previous described. One thinned layer sealant (Teethmate F-1, Kuraray Co. Ltd. Japan) was performed to provide a uniform surface area to which the sealant core will be applied onto the etched enamel. Then bonding cores were taken as 1.5 mm each side to midline parallel to the long axis of the former tooth.

A micro tygon tubing with an internal diameter of 0.8 mm and a height of 2 mm was mounted on sealant-enamel surface. A sealant was filled into the cylinder and cured. Then the tygon tube was removed. After storage under water at room temperature for 24 h, the specimens were tested for micro-shear bond strength using the Universal testing machine (EZ test, Shimadzu Co., Kyoto, Japan). A shear force was applied to each specimen at a cross-head speed of 1 mm/min until failure occured.

After testing, all the fractured surfaces were observed using a SEM to determine the mode of fracture according to fracture position. The fractured specimens were classified into one of six categories as follows:

A- Adhesive failure (AF) in more than 95% of bonded area between enamel and sealant.

B- Cohesive failure (CF) in enamel more than 95% of area.

C- CF in sealing material more than 95% of area.

D- Mixed failure, AF in more than 50% of area.

E- Mixed failure, CF in enamel more than 50% of area.

F- Mixed failure, CF in sealing material more than 50% of area.

A paired t-test was applied to the amassed data.

Effect of moisture challenge on the sealant microleakage.

Forty molars and premolars were collected. The occlusal surface was prepared with modified etching technique as described previously and devided into 2 groups.

- Group 1:

• Subgroup 1a:

On the half of each specimen, sealant was applied on the pit and fissure in the ambient condition (28ºC, 75% relative humidity). Light cured.

• Subgroup 1b:

The rest of fissures was obtained in the oral condition of the healthy volunteer. The specimen was placed at the site of the first mandibular molar for 15 s before applying sealant. Mouth breathing was enforced by holding the volunteer‘s nose.

- Group 2:

• Subgroup 2a:

The sealant was applied on the half of each sample in intraoral condition similar subgroup 1.

• Subgroup 2b:

On the rest of fissures, after placing in intraoral of the volunteer for 15 s, one layer of Prompt L-Pop® (3M, USA) was done following the manufacturer’s recommandation. Cured with a light curing unit for 3 s. The sealant then was applied finally.

All specimens were challenged in thermocycling then immersed in 1% methylene blue in order to performe microleakage examination similar the procedure previous described as microleakage part 1.

Data collected was subjected to nonparametric test for two related samples in each group and for two independent samples between two groups.

Ⅲ. Results

Effect of dye-guidance brushing etching technique on the microleakage of sealant.

The microleakage scores at the interfaces between the three different types of treatments are listed in the Table 1. Statistically, the conventional technique was found to show significantly more microleakage than others (P<.05). But there was no significant difference between the modified etching technique and the enameloplasty group.

Table 1. Microleakage for different treament group (sections)

Group	Number of section (%)				
	0	1	2	3	Total
Modified technique	111 (88.1 %)	6 (4.8%)	0 (0%)	9 (7.1 %)	126 (100%)
Enameloplasty	100 (85.5%)	6 (5.1%)	6 (5.1%)	5 (4.3%)	117 (100%)
Conventional technique	74 (58.3%)	19 (15%)	13 (3.5%)	27 (21.3 %)	127 (100%)

- Groups connected by a line are significant different (P<.05).

As shown in Table 2, the enameloplasty was found to show significantly more penetration behavior than the others (P<.05). But there was no significant difference between the modified etching technique and conventional group.

Table 2. Penetration for different treament group (sections)

Group	Number of section (%) 1	2	3	Total
Modified technique	7 (5.6%)	25 (19.8 %)	94 (74.6 %)	126 (100%)
Enameloplasty	0 (0 %)	10 (8.5 %)	107 (91.5 %)	117 (100%)
Conventional technique	4 (3%)	24 (18.9 %)	99 (78 %)	127 (100%)

- Groups connected by a line are significant different (P<.05).

Effect of modified etching procedure compared with conventional etching method which was directly applied on the surface of fissure.

There was considerable difference between the etch-patterns achieved on the surface of fissure. There was significantly difference between two groups at the bottom of fissure (P<.05) (Table 3).

The variations in the degree of definition attained are less significant. However, some distinct trends were noted. Of particular note is the high prevalence of "pitted defined" etch. This is contrary to the classical, clearly defined etch (type 1 and type 2) commonly illustrated in text book. It appears that the debris or remnant at the bottom area was very difficult to remove even directly vigorous brushing combination with acid.

Table 3: Results of etched pattern on the fissure surface

Group		Quantity[§] (%)	Quality[§§] (%)			
			1	2	3	4
Modified technique	Orifice site (n=20)	100	15.8	84.2	0.0	0.0
	Bottom (n=20)	84.6	5.75	78.88	0.0	15.37
Conventional technique	Orifice site (n=20)	99.75	6.75	93	0.25	0.0
	Bottom (n=20)	59.125	7.5	51.65	1.25	39.6

- Groups connected by a line are significant different ($P<.05$).
- [§]Total % area affected by etchant in each group at orifice or wall area (the well and pitted etched areas).
- [§§]% area occupied by 1: Well etched enamel; 2: pitted etched enamel ; 3: unetched enamel (smooth) and 4: uncleaned enamel.

Effect of modified etching technique on the penetration of sealant in replica study.

Table 4 showed that the length of central site in modified etching technique was significantly difference to one of the conventional group ($P<.05$).

Table 4. Dimensions of matched pits and fissures replica in premolars (mm)

Group	Central point length (Mean ± SD)	Lateral point length (Mean ± SD)
Modified technique (n=11)	0.6073 ± 0.21	0.5564 ± 0.28
Conventional technique (n=11)	0.4536 ± 0.24	0.6163 ± 0.19

- Groups connected by a line are significant different ($P<.05$).

Effect of modified etching technique on the bond strength of sealant on the etched enamel compared with conventional etching method.

Table 5. Micro-shear bond strengths (mSBS) and failure modes (n=35/group)

Group	Mean mSBS ± SD (MPa)	Failure mode[§]						
		a	b	c	d	e	f	Total
Modified technique	7.365 ± 2.08	0	0	18	1	3	13	35
Conventional technique	6.626 ± 1.756	0	1	24	0	2	8	35

- Groups connected by a line are significant different (*P*<.05).
- [§]a: AF >95% bonded area; b: CF in enamel >95%; c: CF in resin >95%; d: Mixed, AF >50%; e: Mixed, CF in enamel >50%; f: Mixed, CF in resin >50%.

The Table 5 presented the mSBS results for the two different testing conditions. The modified etching group was significantly difference than the conventional group (*P*<.05); however, no difference in failure modes between them. Most of failure sites were cohesive failure in resin. The next site was cohesive failure in enamel. There was no adhesive failure at sealant-enamel interface (bonding site).

Effect of moisture challenge on the sealant microleakage.

As regard with the microleakage of sealant applied in moisture condition, there was a significant difference among them (*P*<.05) except sealant applied in oral condition (subgroup 1b and 2a) in both groups (Table 6). The intraoral environment produced more microleakage whatever the material was used. The hydrophilic character of single-bonding agent did not prevent the microleakage of sealant.

Table 6. Percentage of section that showed microleakage.

Group	Moisture condition	Section (%)
Group 1	Subgroup 1a (Sealant/ambient)	3 (5.3%)
(n=38)	Subgroup 1b (Sealant/intraoral)	26 (68.5%)
Group 2	Subgroup 2a (Sealant/intraoral)	23 (59.8%)
(n=42)	Subgroup 2b (Sealant + PLP/intraoral)	11 (26.3%)

- Groups connected by a line are significant different ($P<.05$).

Ⅳ. Discussion

In an vitro study of the penetration abilities of liquid and gel-type etching agent, the results showed that etchants could not penetrate further than the fissure entrances even if the fissures were free of deposits[6)]. Other studies reported that both gel and liquid etchants have the same patterns in penetrating the fissures; however, the presence of debris in the fissures impeded the complete penetration of etchants[7)]. Similar findings related to insufficient penetration of etching agents and lack of sealant adaptation in the fissures have been reported by different investigators[8,9)].

The penetration of liquids in narrow capillaries is influenced by the properties of the liquid, such as viscosity, surface tension and the surface free energy of the capillary wall[7)]. Many studies proved that the addition of surfactant to a etchant lowers its surface tension and thereby improves the performance of sealing materials[10,11)]. In this present study, no surfactant was used. Thus the less microleakage in experimental group (Table 1) could be explained by the effect of brushing action.

The role of active (or dynamic) appplication may be seen by observing the etching patterns of the enamel. From the SEM observations of enamel surfaces, etched with active application, the surface debris was removed and the enamel etching pattern was more evident compared to those of inactive application (Table 3). The function of active application is to achieve micromechanical interaction with the underlying enamel.

Hydroxyapatite dissolution during phosphoric acid application proceeds rapidly initially, but is eventually impeded by the precipitation of monocalcium phosphate monohydrate ($Ca(H_2PO_4)_2.H_2O$) on the enamel surface[12]. This calcific debris could conceivably interfere with proper sealant penetration by occluding the enamel micropores. This phenomenon was one reason for the recommendation of acid application by brushing. It is believed that the agitation resulting from the brushing action will disrupt the precipitate and continually expose the underlying enamel to dissolution. In the micro-shear bond test, the specimens which were prepared on enamel surfaces to which the acid was applied but not agitated were showed less bond strengths than those of specimens prepared on enamel surfaces to which the acid was applied by a brushing action (Table 5). This finding was supported by a recent study reported that etchant application with a light brushing motion could significantly decrease microleakage[13].

Agitation may be achieved by stroking, dabbing or rubbing. In the study of Oliver[14], a stroking action was used, which implied a light action and it was found that generally this method of agitation leaded to a higher score, but little change in etch type. In this present study, similar changes also were found (Table 3). Bates et al.[15] also found that etch type was indistinguishable between a dabbing action and no agitation. Rubbing action was found by both Bates et al.[15], and Hormati et al.[16], to obscure the prism boundaries and give more uniform etch.

On the other hand, a continuous but gentle dabbing or agitation of the etchant agent on the enamel surface, in contrast to rubbing, had been recommended because it preserves the maximal length of the delicate enamel prism sheath[17,18] or decreased of microleakage[19]. Although dabbing the etching solution has been shown to improve the microscopic quality of the fragile surface topography, further investigation has failed to demonstrate a difference in either tensile[15] or shear[16] bond strengths for the applied sealants regardless of the application method tested. Hormati et al.[16]showed that the etched pattern created by the rubbing technique is not as sharp as the normal etched pattern and that prism peripheries (prism sheaths) are shorter and more blunted. And rubbing the cotton pledget on the enamel surface during the etching process may disrupt the normal pathway of etching. However, their results were not found in this present study. The present finding proved that the fragile etched enamel pattern (Table 3) and micro-shear bond strength (Table 5) were not influenced in this suggested manner. These findings could be explained by the extended non-agitated time after brushing action.

In addtion, Retief[20] showed that the rate of etching of enamel by 50% phosphoric acid became more or less constant after 15 s had elapsed. In the first 15 seconds the enamel was etched more slowly, which may involve removal of a superficial layer different from the underlying enamel.

This present finding was different with result of Garcia-Godoy et al.[21] who used a dental explorer to tease the acid etchant into the fissures. It is assumed that the different results due to the different size of dental explorer (Φ=0.2 mm) and the very fine fibre (Φ= 0.06 mm) of microbrush.

In this present study, the prophylaxis with pumice was not applied in testing group, only vigorous brushing with etchant agents. However the result did suggest that the microleakage of sealant was significantly lower in the samples in the test group compared to the conventional group (with prophylaxis) (Table 1).

A lot of studies suggested that a rotating bristle brush with pumice paste is unlikely to clean pellicle from fissure embrasures because the bristles sweep across the inaccessible regions and polish only the more exposed superficial areas[22,23]. In addition, traces of pumice particles may be found in the depth of the fissures; these may interfere with acid etching and be incorporated into the sealant resin. However some recent studies showed that the pumicing was still high value[24,25].

Various other cleaning methods have been tested. Air polishing with the Prophy-Jet followed by acid etching produced the highest tensile bond strength of all groups tested[26]. Similarly, air polishing combined with acid etching resulted in an improved surface for resin wetting as determined by the number of resin tags formed[27]. Another study on fissure preparation prior to sealant application found that the air-polishing system performed well. Fissure cleaning with air-polishing unit produced a statistically significant increase in depth of penetration of sealant resin, and its use as a standard cleaning method before fissure sealing was recommended[28]. In other report, the air-polishing method showed the least degree of microleakage of the sealants of all prophylaxis methods[29]. Similar results were found by other authors who used air-abrasion technique[30]. However air polishing and air abrasion prior to etching never really became the standard for pit and fissure sealant application procedures, possibly due to the increase in equipment cost and complexity of the procedure.

A number of authors have looked at other more aggressive methods of fissure preparation prior to sealant application: the enameloplasty technique. Despite the positive results of this method, only a small number of short-term clinical studies with small samples support this technique as equal to, but not better than, sealant placement without enameloplasty[31,32]. Feigal[33] stated that universal use of fissure eradication or enameloplasty with rotary instruments or air-abrasion is an unnecessary addition to good sealant methodology and the enameloplasty procedure itself may injure normal enamel resulting in higher caries susceptibility of that fissure in the future. In addition, recent study showed that neither air abrasion nor enameloplasty followed by acid etching produced significantly less microleakage than the traditional pumice prophylaxis with acid etching technique[34]. They recommended that the routine removal of healthy sound tooth structure (air abrade or mechanical technique) prior to sealant placement does not seem justified.

In the present study, result showed that the similar leakage after opening the fissures with a fine-grit diamond bur followed by acid etching compared with brushing etching. This finding followed the results of other author[35]. They suggested that a bigger volume of sealant material and therefore higher polymerization shrinkage forces occur that stress the enamel-resin-interface. In this present study, only one kind of bur was used, so this finding cannot extrapolate to other kinds of bur (very fine-grit or carbide bur…).

In addition, the standardized technique in enameloplasty depends on the practician's experience. Halterman et al.[36] found that instrumentation varied and a large range of depths were used. According to their results, 50% of pediatric dentists always used a light "sweep" of the grooves (<0.5 mm) without necessarily removing all staining or "chalkiness" in the grooves. Similar with their findings, in this present study, remained remnants were found in the deepest of fissures in the enameloplasty group although it was performed by experience dentist.

On the other hand, many reports have described arrested caries and the elimination of viable microorganisms under sealants or restorations with sealed margins[37,38]. So, careful cleaning of the enamel surface and the fissure followed by effective etching of the fissure walls will result in a successful sealant and will halt progression of any existing incipient caries.

Many studies showed that non-etched areas at the base of fissures were commonly found regardless of the type of etchant[7,39]. In the direct etched study, debris still remained, specially at the bottom side, although the wall of the fissure was directly brushed and etched (Table 3). However the microleakage in the test group was not different with one of the enameloplasty group (Table 1). It suggested that the penetration of etchant agent was not directly proportional to the effect of acid, specially at pit and fissure side. Ripa[40] considered that as long as the sealant remained intact, caries should not develop beneath it. At least the cuspal inclined planes across the fissure orifice should be completely filled with sealant[6].

An other technique was introduced, vibration preparation. Tadokoro et al.[41] used vibration-etching for fissure sealant application. The vibration-etching mechanically removed the fissure contents of film and enlarged the upper portion of the fissures. However, the sealant failed to penetrate the narrowest spaces, and the fissure contents were sometimes found incorporated in the sealant. Their report stated that penetration seemed to be prevented not only by presence of the fissure content but also by an insufficient wetting effect. An other previous study showed that there were no statistically significant differences in microleakage and penetration ability between the etching procedure with a vibration system (Intralux prepcontrol head) compared to that with a light brushing motion. And the authors concluded that the need of the etching with a vibration technique requiring a special device might not be necessary when the practical etching procedure with a brushing motion yielded the similar encouraging results in decreasing the leakage[13]. Of the same result, recent study showed that sealant penetration was more influenced by the shape, depth and residual debris of fissures than by use of a vibration probe (Microvibe unit). And there were no significant differences between the non-vibration groups and vibration groups[42].

Sealant materials do not simply bond to the enamel surface but actually penetrate into the microposorities created in the surface enamel during the etching procedure. Infiltration of the etched enamel results in formation of resin tags, which provide the mechanical means for sealant retention. But recent study showed that the depth of resin penetration into the etched enamel surfaces is not of prime importance[43,44]. Nakabayashi et al.[45] suggested that the resin-enamel bond strength is the result of the cumulative cross-sectional area of resin infiltrating the enamel space. Recent studies confirmed the value of enamel hybrid layer. They proved that no difference in resin-enamel bond strengths between prismatic and aprismatic enamel after phosphoric acid etching[46,47]. In this present study, although the tag or the typical etched enamel pattern was rarely found (Table 3), the low microleakage still existed specially in the modified group. This finding suggested that the pitted etched enamel may play an important role in the performance of sealing material (Fig. 4B, 4C).

The finding that the cohesive failure in resin-enamel interface was not found can be explained by another reason (Table 5, Fig. 5). It has been proposed that the methacrylates with hydrophilic and hydrophobic groups promote the diffusion of monomers due to their good affinity for tissues. MDP (10-Methacryloyloxydecyl Dihydrogen) in Teethmate F-1 was essential in promoting penetration and bonding by the formation of a resin-infiltrated enamel and resin tags in the subsurface of enamel. In addition, self-etching effect of MDP improved the penetration, and Teethmate F-1 appeared to exceed the subsurface etched depth[48].

The present finding of increase microleakage of sealant in intraoral condition (mouth breathing) whatever materials used were interesting (Table 6). This finding is not in accordance with previous work showed that the use of bonding agent (in that study was Optibond system) as a sealing material under moisture contamination exhibited no significant increase in microleakage compared to the use of sealant alone (Concise)[49]. In the present study, although the specimen was placed only for 15s in simulated mouth breathing group, the voids were found along the interface between the enamel surface and the sealant (Fig. 6B). This finding could be explained by the osmotic blistering related with single-step bonding agent that was described in recent study[50]. And the relative increasing microleakage profile in single-step bonding agent group (Table 6) was probably due to this osmotic blistering.

The oral condition may be considered a moist environment. However, in mouth breathing, it is the moisture content of the air. Hence the effect of moisture is not only related to the surface moisture of the etched enamel, but also the effect it may have on the resinous materials as well.

The work by Plasmans et al.[51)] showed that air humidity was a detrimental factor in dentine adhesion. They indicated a large decrease in bond strength for Scotchbond Multi-Purpose. It is believed that a relative humidity over 90% will result in rapid condensation of water on the tooth surface. This is most meaningful to those dentists who still do not use the rubber dam during restorative dental procedures[52)]. Plasmans et al.[53)] found clinically that intraoral conditions are cyclical, and fluctuate as the patient inhales and exhales. They noted that a ‘dry’ field in the oral cavity cannot be gained without the application of a rubber dam. The results of the current study, along with other authors[53,54)], confirmed that conditions of humidity and temperature could influence on the success of sealant placement.

At last, the current study showed that the use of plaque detector dye before pit and fissure etching did not increase microleakage (Table 1, Fig. 4A). This suggested that detector dye can be useful as a remnant indicator to aid the dentist in preparation of pit and fissure.

V. Conclusion

The purpose of this in vitro study was to examine the effects of dye-guidance brushing etching method on the performance of pits and fissures sealant using microleakage test, direct fissure surface etched pattern experiment, replica study, and micro-shear bond strength testing observation. In addition, the influence of moist challenge on the microleakage of sealant was examined. The results of present study can be summarized as follows:

- The microleakage of testing group was significant statistically different with conventional method ($P<.05$) and was not different with the enameloplasty group ($P>.05$).
- The quality and quantity of etched pattern were improved by the suggested method.
- Micro-shear bond strength of testing group was higher than conventional group ($P<.05$).
- The environmental condition was influenced on the performance of the sealant.

Reference

1. Raadal M, Espelid I, Mejare I : The caries lesion and its management in children and adolescents. In: Pediatric Dentistry: a clinical approach. Koch G, Poulsen S (eds). Copenhagen: Munksgaard, pp 173-212, 2001.
2. Harris NO, Garcia-Godoy F : Primary preventive dentistry. 5th edtion. London: Asimon and Schuster Company, 1999.
3. Waggoner WF, Siegal M : Pit and fissure sealant application: updating the technique. J Am Dent Assoc, 127:351-61, 1996.
4. Shapira J, Eidelman E : Six-year clinical evaluation of fissure sealants placed after mechanical preparation: a matched pair study. Pediatr Dent, 8:204-5, 1986.
5. Lygidakis NA, Oulis KI, Christodoulidis A : Evaluation of fissure sealants retention following four different isolation and surface preparation techniques: four years clinical trial. J Clin Pediatr Dent, 19:23-5, 1994.
6. Garcia-Godoy F, Gwinnett AJ : Penetration of acid solution and gel in occlusal fissures. J Am Dent Assoc, 114:809-810, 1987.
7. Brown MR, Foreman FJ, Burgess JO, et al. : Penetration of gel and solution etchants in occlusal fissures. ASDC J Dent Child, 55:265-8, 1988.
8. Taylor CL, Gwinnett AJ :A study of the penetration of sealants into pits and fissures. J Am Dent Assoc, 87:1181-8, 1973.
9. Symons AL, Chu CY, Meyers IA : The effect of fissure morphology and pretreatment of the enamel surface on penetration and adhesion of fissure sealants. J Oral Rehabil, 23:791-8, 1996.
10. Bottenberg P, Graber HG, Lampert F : Penetration of etching agents and its

influence on sealer penetration into fissures in vitro. Dent Mate,. 12:96-102, 1996.

11. Gungor HC, Altay N, Batirbaygil Y, et al. : In vitro evaluation of the effect of a surfactant-containing experimental acid gel on sealant microleakage. Quintessence Int, 33:679-84, 2002.

12. Chow LC, Brown WE : Phosphoric acid conditioning of teeth for pit and fissure sealants. J Dent Res, 52:1158, 1973.

13. Duangthip D, Lussi A : Effects of fissure cleaning methods, drying agents, and fissure morphology on microleakage and penetration ability of sealants in vitro. Pediatr Dent, 25:527-33, 2003.

14. Oliver RG : The effect of different methods of bracket removal on the amount of residual adhesive. Am J Orthod Dentofacial Orthop, 93:196-200, 1988.

15. Bates D, Retief DH, Jamison HC, et al. : Effects of acid etch parameters on enamel topography and composite resin--enamel bond strength. Pediatr Dent, 4:106-10, 1982.

16. Hormati AA, Denehy GE, Fuller JL : Retentiveness of enamel-resin bonds using unfilled and filled resins. J Prosthet Dent, 47:502-4, 1982.

17. Tyler JE : A scanning electron microscope study of factors influencing etch patterns of human enamel. Arch Oral Biol, 21:765-9, 1976.

18. Silverstone LM : Fissure sealants: the enamel-resin interface. J Public Health Dent, 43:205-15. 1983

19. Speiser AM, Segat TE : The influence of technique modifications on sealant leakage. ASDC J Dent Child, 47:93-6, 1980.

20. Retief DH : A comparative study of three etching solutions. Effects on contact

angle, rate of etching and tensile bond strength. J Oral Rehabil, 1:381-90, 1974.

21. Garcia-Godoy F, Gwinnett AJ : An SEM study of fissure surfaces conditioned with a scraping technique. Clin Prev Dent, 9:9-13, 1987.
22. Donnan MF, Ball IA : A double-blind clinical trial to determine the importance of pumice prophylaxis on fissure sealant retention. Br Dent J, 165:283-6, 1988.
23. Burrow MF, Makinson OF : Pits and fissures: remnant organic debris after acidetching. ASDC J Dent Child, 57:348-51, 1990.
24. Chevitarese AB, Chevitarese O, de Souza IP, et al. : Influence of prophylaxis on the microleakage of sealants: in vitro study. J Clin Pediatr Dent,26:371-376, 2002.
25. Ansari G, Oloomi K, Eslami B : Microleakage assessment of pit and fissure sealant with and without the use of pumice prophylaxis. Int J Paediatr Dent, 14:272-278, 2004.
26. De Craene GP, Martens LC, Dermaut LR : A clinical evaluation of a light-cured fissure sealant (Helioseal). ASDC J Dent Child, 56:97-102, 1989.
27. Brockmann SL, Scott RL, Eick JD : A scanning electron microscopic study of the effect of air polishing on the enamel-sealant surface. Quintessence Int, 21:201-6,1990.
28. Brocklehurst PR, Joshi RI, Northeast SE : The effect of air-polishing occlusal surfaces on the penetration of fissures by a sealant. Int J Paediatr Dent, 2:157-62, 1992.
29. Lee HG, Lee SH : A study on the microleakage of sealants according to the prophylaxis methods. J Korean Acad Pediatr Dent, 22: 59-72, 1995.
30. Ellis RW, Latta MA, Westerman GH : Effect of air abrasion and acid etching

on sealant retention: an in vitro study. Pediatr Dent, 21:316-31, 1999.

31. Le Bell Y, Forsten L : Sealing of preventively enlarged fissures. Acta Odontol Scand,38:101-4,1980.

32. Shapira J, Eidelman E : Six-year clinical evaluation of fissure sealants placed after mechanical preparation: a matched pair study. Pediatr Dent, 8:204-5, 1986.

33. Feigal RJ : The use of pit and fissure sealants. Pediatr Dent, 24:415-422, 2002.

34. Blackwood JA, Dilley DC, Roberts MW, et al. : Evaluation of pumice, fissure enameloplasty and air abrasion on sealant microleakage. Pediatr Dent, 24:199-203, 2002.

35. Manhart J, Huth KC, Chen HY, et al. : Influence of the pretreatment of occlusal pits and fissures on the retention of a fissure sealant. Am J Dent, 17:12-8, 2004.

36. Halterman CW, Rayman M, Rabbach V : Survey of pediatric dentists concerning dental sealants. Pediatr Dent, 17:455-6, 1995.

37. Kramer PF, Zelante F, Simionato MR :The immediate and long-term effects of invasive and noninvasive pit and fissure sealing techniques on the microflora in occlusal fissures of human teeth. Pediatr Dent, 16:108-112, 1993.

38. Mertz-Fairhurst EJ, Adair SM, Sams DR, et al. : Cariostatic and ultraconservative sealed restorations: nine-year results among children and adults. ASDC J Dent Child, 62:97-107, 1995.

39. Garcia-Godoy F, Malone WF : Penetration of acid gel etchants into cavity walls: an SEM evaluation. Quintessence Int, 18:481-5, 1987.

40. Ripa LW : The current status of pit and fissure sealants. J Can Dent Assoc, 51:367-380, 1985.

41. Tadokoro Y, Iwaku M, Fusayama T : A laboratory report on vibration etching for fissure sealants. J Dent Res, 61:780-785, 1982.
42. Hosoya Y, Garcia-Godoy F, Summitt JB : Microleakage and sealant penetration using a vibrating probe. Am J Dent, 17:427-32, 2004.
43. Legler LR, Retief DH, Bradley EL : Effects of phosphoric acid concentration and etch duration on enamel depth of etch: an in vitro study. Am J Orthod Dentofacial Orthop, 98:154-60, 1990.
44. Hobson RS, McCabe JF, Rugg-Gunn AJ : The relationship between acid-etch patterns and bond survival in vivo. Am J Orthod Dentofacial Orthop, 121:502-9, 2002.
45. Nakabayashi N et al. : Hybridization of dental hard tissue. Tokyo: Quintessence, pp 88-99, 1998.
46. Kanemura N, Sano H, Tagami J : Tensile bond strength to and SEM evaluation of ground and intact enamel surfaces. J Dent, 27:523-30, 1999.
47. Ibarra G, Vargas MA, Armstrong SR, et al. : Microtensile bond strength of self-etching adhesives to ground and unground enamel. J Adhes Dent, 4:115-24, 2002.
48. Irinoda Y, Matsumura Y, Kito H, et al. : Effect of sealant viscosity on the penetration of resin into etched human enamel. Oper Dent, 25:274-82, 2000.
49. Duangthip D, Lussi A : Microleakage and penetration ability of resin sealant versus bonding system when applied following contamination. Pediatr Dent, 25:505-11, 2003.
50. Tay FR, Lai CN, Chersoni S, et al. : Osmotic blistering in enamel bonded with one-step self-etch adhesives. J Dent Res, 83:290-5, 2004.
51. Plasmans PJ, Reukers EA, Vollenbrock-Kuipers L, et al. : Air humidity: a detrimental factor in dentine adhesion. J Dent, 21:228-33, 1993.

52. Hagge MS, Pierson WP, Mayhew RB, et al. : Use of rubber dam among general dentists in the United States Air Force dental service. Oper Dent, 9:122-9, 1984.
53. Plasmans PJ, Creugers NH, Hermsen RJ, et al. : Intraoral humidity during operative procedures. J Dent, 22:89-91, 1994.
54. Nystrom GP, Holtan JR, Phelps RA 2nd, et al. : Temperature and humidity effects on bond strength of a dentinal adhesive. Oper Dent, 23:138-43, 1998.

Explanation of Figures

Fig. 1. Flow chart of microleakage test.

Fig. 2. Etched pattern score. 1: Well defined; 2: Poorly defined (pitted etched pattern); 3: Unetched; and 4: Uncleaned.

Fig. 3. Micro-shear bond test.

Fig. 4. Adaptation in modified group: (A) Sealant (s) adapted well to fissure wall. Residual debris (d) remained in deepest portion of fissure (stained by plaque detector before etching). e: enamel. (B). SEM micrograph of another demineralized specimen taken from the deep part of fissure. A thin hybrid layer (h) was formed along the superficial aprismatic enamel (ap). s: sealant, p: prismatic enamel. (C). SEM micrograph of the enamel surface of fissure wall presented pitted etched pattern.

Fig. 5. Most of failure modes were cohesive failure (cf) in material. ef: enamel failure .

Fig. 6. Adaptation in moist challenge group: (A). Gap (g) of sealant. (B). Osmotic blister (arrows) in single-step adhesive group. s: sealant, e: enamel.

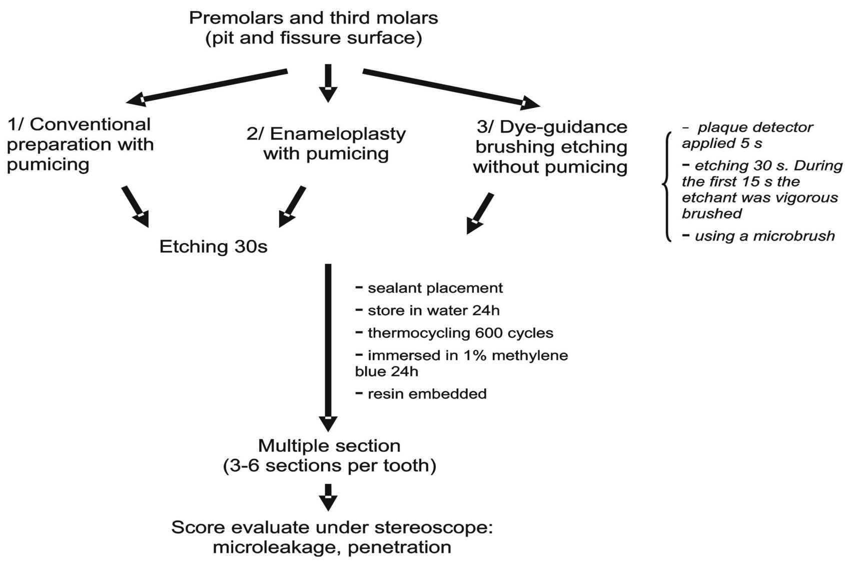

Fig. 1. Flow chart of microleakage test.

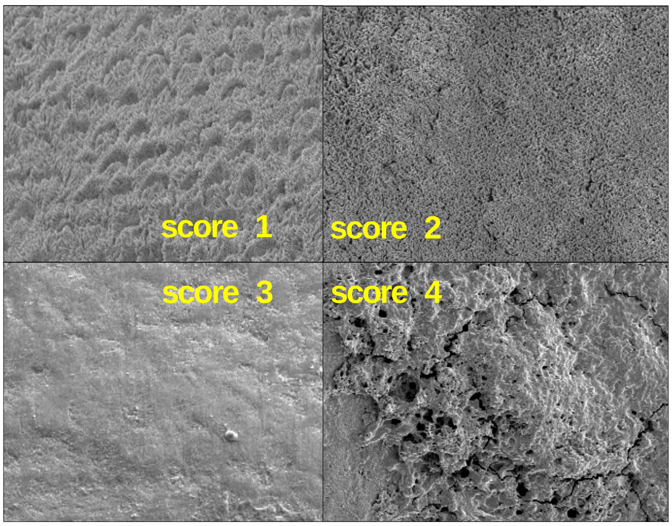

Fig. 2. Etched pattern score.

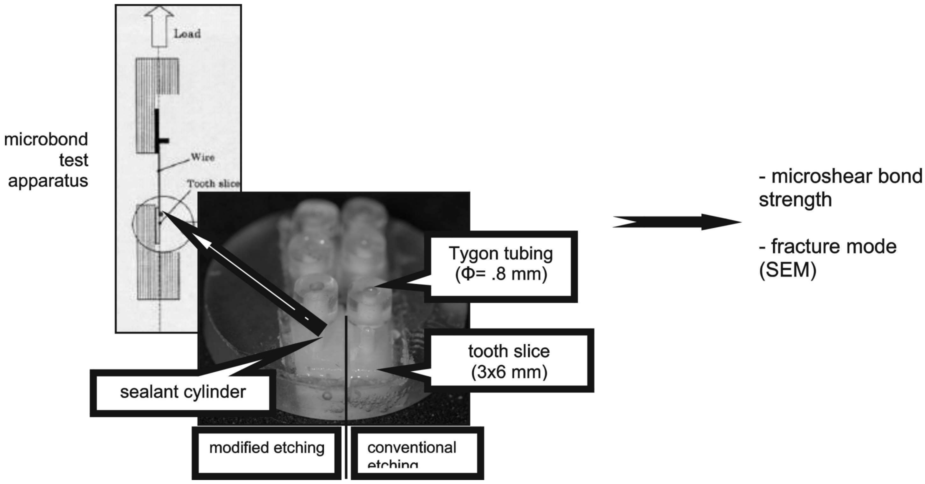

Fig. 3. Micro-shear bond test.

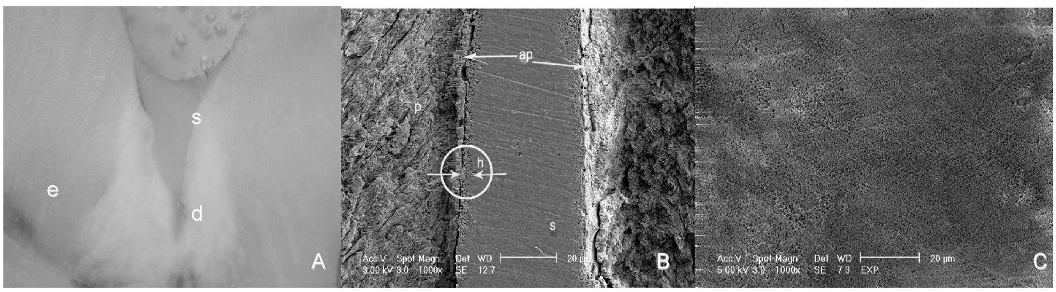

Fig. 4. Adaptation in modified group.

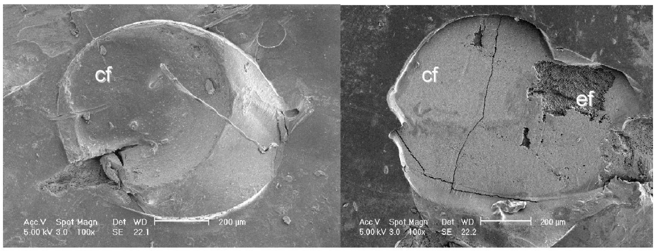

Fig. 5. Failure mode.

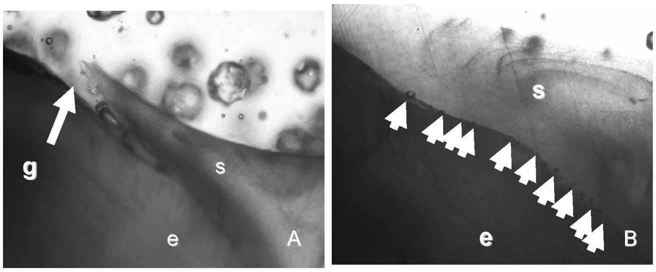

Fig. 6. Adaptation in intra-oral group.