A Study on Fissure Penetration and Microleakage of Sealants after Preparations of Occlusal Pits and Fissures

Ji Young Chung, D.D.S

Department of Dentistry,

The Graduate School,

Yonsei University

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Ji Young Chung, D.D.S.

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This certifies that the masters thesis of

Ji Young Chung is approved.

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Thesis Supervisor: Prof. Sohn, Heung Kyu

_____

Thesis Committee Member: Prof. Choi, Byung Jai

_____

Thesis Committee Member: Prof. Lee, Chan Young

The Graduate School

Yonsei University

June 2002

감사의 글

뒤늦게 시작하여 마침내 작은 결실을 맺을 수 있게 해주신 하느님께 감사 드립니다.

논문을 완성하기까지 연구의 지도교수로서 귀한 가르침을 주신 손홍규 교수님께 깊은 감사를 드리며, 바쁘신 가운데서도 심사를 맡아 자문하여 주신 최병재 교수님, 이찬영 교수님, 또 이종갑 선생님, 최형준 선생님께도 감사 드립니다. 실험을 배려해 주시고 많은 조언을 아끼지 않으셨던 해부학교실의 김희진 교수님께도 감사 드리며 늘 기꺼이 도움을 주신 강민규 선생님을 비롯한 해부학 교실원 및 소아치과 의국원 여러분, 꼼꼼히 마지막 교정을 해주신 김성오 선생님께도 감사한 마음을 전하고 싶습니다.

처음부터 격려를 아끼지 않은 박이화 선생과 연세덴티프로 치과원장들과도 함께 기쁨을 나누고 싶습니다.

마지막으로 오늘이 있기까지 따뜻한 사랑으로 돌보아 주시고 격려해 주신 부모님께, 또 넓은 아량으로 이해해 주신 시부모님과 마무리작업을 도와준 시동생 성한씨에게도 깊이 감사 드리며 멀리서 응원해준 사랑하는 남편 성호씨와 아들 호진, 유진에게 감사함과 미안한 마음을 함께 전하고 싶습니다.

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저자 씀

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ABSTRACT

A Study on Fissure Penetration and Microleakage of Sealants after Preparations of Occlusal Pits and Fissures

Ji Young Chung, D.D.S.

Department of Dentistry, The Graduate School, Yonsei University

(Directed by Prof. Heung Kyu Sohn, D.D.S., M.S. and Ph.D.)

Objective: Considering the importance of sealant integrity, the purposes of this study were as follows.

1. To compare the fissure penetration and microleakage of sealants after acid etching, bur preparation with acid etching and air abrasion preparation with acid etching.
2. To compare the fissure penetration and microleakage of filled and unfilled sealants using the same three preparation methods.

Materials and Methods: Seventy-two freshly extracted human premolars for orthodontic purposes with no or minimal occlusal caries were assigned to six groups. In group 1 and 2, teeth were prepared using acid etching only. In group 3 and 4, the pits and fissures were opened with 1/4 round carbide bur in a high speed handpiece to an approximate diameter of the bur followed by

acid etching. In group 5 and 6, teeth were air abraded using a high-velocity air microabrasion machine (Prep. Star®, Danville Inc.). The kinetic cavity preparation technique was performed, using the particle size of 50㎛ aluminum oxide and nozzle tip size of 0.15/80° . In group 1, 3 and 5, a filled sealant (Ultraseal XT *plus*®, Ultradent, U.S.A) was used with a drying agent (Primadry®, Ultradent). In group 2, 4 and 6, teeth were sealed with an unfilled sealant (Teethmate F-1®, Kuraray, Japan). After application and curing of sealants, all teeth were thermo-cycled between 5℃ and 55℃ for 1200 cycles. And then, the teeth were dried and coated with two coats of nail varnish and then immersed in a 5% methylene blue solution for 24 hours. Each tooth was sectioned buccolingually parallel to their axes. These sections were examined under a stereo microscope and all images were computerized to evaluate the degree of fissure penetration and microleakage. Statistical analysis was completed using Duncan's test and Tukey's test.

Results:

1. Superior results in both sealant penetration and microleakage were shown when the tooth surface was prepared by a bur (group 3 & 4). ($P<0.05$)
2. The sealant penetration rate was significantly higher in air

abrasion groups (group 5 & 6) than acid etching groups (group 1 & 2). ($P<0.05$)

3. Acid etching only groups (group 1 & 2) and air abrasion groups(group 5 & 6) yielded similar results in microleakage. ($P>0.05$)

4. No statistically significant difference in the penetration and microleakage was found between filled and unfilled sealants. ($P>0.05$)

Key words : preparation of occlusal pits and fissures, pit and fissure sealants, fissure penetration, microleakage

Ⅰ. INTRODUCTION

Ji Young Chung, D.D.S.

Department of Dentistry, The Graduate School, Yonsei University

(Directed by Prof. Heung Kyu Sohn, D.D.S., M.S. and Ph.D.)

Pits and fissures are generally considered as incompletely fused forms of enamel during cupsal odontogenesis. As a result, pits and fissures have narrow, deep and irregular morphology and they have been described as the single most important feature leading to the development of occlusal caries.[39] Preventive techniques such as systemic and topical fluorides are thought to preferentially protect smooth rather than occlusal surfaces.[22] The prevalence of caries on pit and fissure surfaces emphasizes the importance of sealant in the prevention of caries today.[36]

Pit and fissure sealant has been accepted as a caries preventive strategy since the 1970s.[2] The effectiveness of sealants hinges on their ability to isolate pits and fissures from the combination of bacteria, their nutrients and acidic metabolic products.[15,18,23] Microl eakage has been defined as the clinically undetectable passage of bacteria, fluids, molecules or ions between the cavity wall and the applied restorative material.[20,28,31] At the sealant-enamel interface,

microleakage may limit the efficacy of sealants by providing a pathway for materials which support the progression of the cariogenic process underneath the sealant.[15,18,23]

A number of studies have examined the influence of tooth preparation on microleakage of pit and fissure sealant. Studies on the conventional method of preparing the enamel surface prior to sealant placement by acid etching alone show varying results although most reported some degree of microleakge.[10,17,27] Microleakage studies examining the effect of opening pits and fissures with a bur before sealant placement also have mixed results. Hatibovic-Kofman *et al.*[19] reported that significantly less microleakage occurred using a bur preparation followed by acid etching compared with conventional acid etching only or air abrasion without etching. Conversely, Xalabarde *et al.*[45] found no significant difference between conventional acid etch only and bur preparation with acid etching of pits and fissures. The results of most studies investigating microleakage after the use of air abrasion to open pits and fissures depended upon whether the enamel was acid etched or not. Most studies have indicated that significantly increased microleakage occured using air abrasion alone without acid etching when compared with acid etching alone or bur preparation with acid etching.[9,19,21,32] Zyskind *et al.* compared preventive resin restorations prepared with air abrasion

or bur both followed by acid etching and found no significant difference in the degree of microleakage.[46] There have been no studies directly comparing the degree of microleakage of several sealants following preparation of pits and fissures with acid etch only, bur preparation with acid etching, and air abrasion preparation with acid etching.

The purposes of this study were ;

1. To compare the fissure penetration and microleakage of sealants after acid etching only, preparation with bur followed by acid etching and air abrasion preparation with acid etching.
2. To compare the fissure penetration and microleakage of filled and unfilled sealants using three methods of tooth preparation.

Ⅱ. MATERIALS AND METHODS

!. Materials

Seventy-two freshly extracted human premolars for orthodontic purposes with no or minimal occlusal caries were stored in saline prior to the experiment. Ultraseal XT *plus*® (Ultradent®, U.S.A), filled sealant with a drying agent and Teethmate F-1® (Kuraray, Japan), an unfilled sealant, were used.

2. Sample preparation

All teeth were cleaned with a nonfluoridated pumice using a rubber cup in a low speed handpiece. The teeth were randomly assigned to six groups of 12 teeth each. The groups are shown in Table 1. In group 1 & 2, teeth were prepared using acid etching only. Acid etching was completed with 35% phosphoric acid gel, left on teeth for 15 seconds, followed by 15 seconds washing and 15 seconds drying. In group 3 & 4, the pits and fissures were opened with 1/4 round carbide bur in a high speed handpiece followed by acid etching. In group 5 & 6, teeth were air abraded using a high-velocity air microabrasion machine (Prep. Star®, Danville Inc.). The kinetic cavity preparation technique was performed, using the particle size of 50㎛ aluminum oxide and

nozzle tip size of 0.15/80° to open the pits and fissures with subsequent acid etching.

In groups 1, 3 and 5, a filled sealant that contained fluoride (Ultraseal XT *plus*®, Ultradent, U.S.A) was used with a drying agent (Primadry®, Ultradent). In groups 2, 4 and 6, teeth were sealed with an unfilled sealant which also contained fluoride (Teethmate F-1®, Kuraray, Japan). After applying sealants, all teeth were light-cured for twenty seconds with a light curing unit. Immediately after curing the sealants, teeth were placed in distilled water.

All teeth were thermo-cycled between 5℃ and 55℃ for 1000 cycles. The dwell time in each bath was 20 seconds. After thermocycling, teeth were embedded in self-curing resin and left for 1 hour for sufficient curing. After that, to prevent the dehydration of the teeth, teeth were stored in saline before immersion in dye.

After the storage in saline, the teeth were dried and coated with two layers of nail varnish and then immersed in a 5% methylene blue solution for 24 hours to allow dye penetration into possible gaps between the tooth substance and the sealant. After removal from the dye, teeth were rinsed with tap water and dried. Two sections were obtained by grinding off the embedded teeth buccolingually parallel to their axes using a

water-cooled diamond disc on an Isomet saw® (Buehler Ltd, USA). Total of 144 sections were obtained. These sections were examined under ×20 magnification with a stereo microscope (Olympus BX 50, Japan) and all images were computerized.

Table 1. Distribution of groups and samples according to various experimental design

Group	Surface Treatment	Sealant Type
1	Acid Etching	Filled Sealant
2	Acid Etching	Unfilled Sealant
3	Bur + Acid Etching	Filled Sealant
4	Bur + Acid Etching	Unfilled Sealant
5	Air Abrasion + Acid Etching	Filled Sealant
6	Air Abrasion + Acid Etching	Unfilled Sealant

3. Evaluation of penetration and microleakage of fissure sealant

Using image evaluation program (Image-Pro plus® ver 3.0, U.S.A.), each section was examined and fissure depth, fissure penetration depth of sealant and dye penetration depth were measured. Fissure penetration rate was determined by the percentage of sealant penetration per fissure depth. Microleakage was determined by the percentage of the depth of dye penetration per fissure penetration of sealant and each value was rated on the dye penetration scale from score of 0 to 3 (Table 2), (Figure 2.1.~2.4.). The extent of sealant penetration and microleakage scores were calculated and analyzed for each group with Duncan's test and Tukey's multiple comparison test using SPSS Version 8.1 Program.

Table 2. Criteria for the degree of microleakage

Score	Extent of dye penetration
0	0 %
1	0 % < score 1 ≤ 10 %
2	10 % < score 2 ≤ 50 %
3	50 % < score 3 ≤ 100 %

(Modified from Överbö and Raadal, 1990)

Ⅲ. RESULTS

A total of 144 sections were examined for fissure penetration and microleakage of pit and fissure sealants.

1. Evaluation of fissure penetration

The result is shown in Table 3. The groups with acid etching method using filled or unfilled sealant (group 1 and 2) showed significantly less penetration into the fissure ($p<0.05$). Among bur preparation and air-abrasion preparation groups (group 3, 4, 5 and 6), there were no statistically significant differences ($p>0.05$) although bur preparation groups showed higher percentage of penetration (Table 4). Between filled and unfilled sealants, there were no significant differences although in all three different fissure preparation groups, unfilled sealant showed slightly higher penetration percentage.

Table 3. Sealant Penetration

Group	% of Fissure penetration
1	82.230
2	84.576
3	96.403
4	97.834
5	92.091
6	94.859

Table 4. Statistical comparison between groups on the sealant penetration

Group	1	2	3	4	5	6
1		-	*	*	*	*
2			*	*	*	*
3				-	-	-
4					-	-
5						-
6						

* : statistically significant difference($p<0.05$)

- : statistically no significant difference($p>0.05$)

2. Evaluation of microleakage

The microleakage score is shown in Table 5 according to the type of sealants and preparation methods. In Figure 1, the percentage of microleakage score is presented for each group. Compared to acid etching groups, statistics showed that bur preparation groups had significantly less microleakage ($p<0.05$). There were, however, no significant differences between air abrasion groups and acid etching groups (Table 6), although air abrasion groups showed lower microleakage scores. No significant difference was found between filled and unfilled sealants.

Table 5. Microleakage Score

Group	Microleakage Degree				Mean Microleakage	Standard Deviation	Sample Size
	0	1	2	3			
1	10	6	5	3	1.167	1.049	24
2	11	6	2	5	1.000	0.904	24
3	18	3	1	2	0.458	0.321	24
4	16	4	3	1	0.583	0.464	24
5	14	4	4	2	0.917	0.733	24
6	13	6	3	2	0.904	0.652	24

Table 6. Statistical comparison between the groups on the microleakage scores

Group	1	2	3	4	5	6
1		-	*	*	-	-
2			*	*	-	-
3				-	*	*
4					*	*
5						-
6						

* : Statistically significant difference ($p<0.05$)

- : Statistically no significant difference ($p>0.05$)

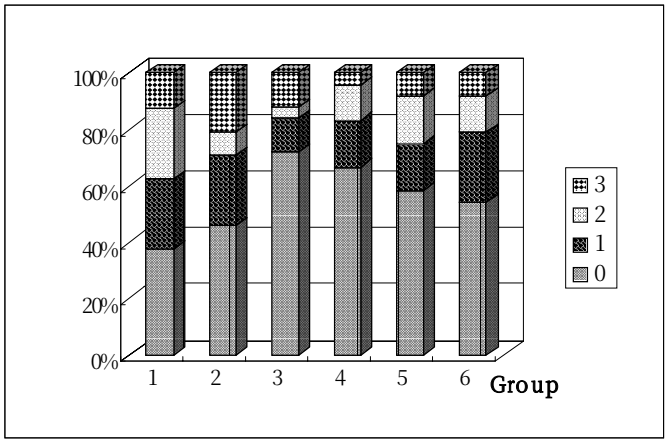

Fig. 1. Bar graph representing the distribution of microleakage scores within each group.

Ⅳ. DISCUSSION

The preventive effect of sealant is gained and maintained only as long as they remain completely intact and bonded in place.[34] And for sealants to be effective, they must also prevent leakage.

For the adequate retention of sealant, it is necessary to maximize the surface area for bonding and that the surface of enamel should be clean, free of salivary contamination and dry at the time of sealant placement.[13,39]

Micromechanical retention for the sealant is provided through surface irregularities created by conditioning the enamel with acid prior to the application of the sealant. However, a previous study[12] showed that even if acid etching gels or solutions are scraped into the fissures with an explorer, the gel, solutions or sealant did not penetrate beyond the region of fissure constriction. Consequently, pit and fissure sealants bond to the cuspal incline planes and not the bottom of the pit and fissures.[8,14] Moreover, questionable carious lesions may cause problems and since sealing of carious fissures cannot be considered an acceptable clinical practice, the use of enameloplasty techniques (fissure enlargement with a bur) has been proposed[8,14], which may improve the bond strength of the sealant, sealant penetration and adaptation to etched enamel. Several studies have evaluated the techniques of

cleansing the fissures to prepare them for etching and sealing.[12,13] The important advantages of this invasive technique are as follows. First, suggested by Shapira[33], this procedure widens and deepens the fissure by eliminating organic material and plaque and a very thin layer of enamel. In addition, Conniff and Hamley[26], in their study on primary teeth, suggested that the retentive strength of the acid-etch bonding system was increased after partial or complete removal of the outermost prismless surface layer. Consequently, these findings lead to the second advantage. It was suggested in some studies that higher retention rates for sealants were obtained following mechanical preparation of the fissure area.[38] According to Tadokoro, the sealant can easily penetrate into the artificially enlarged fissures and adhere to the walls resulting in a better retention. Consequently, there is no need to cover a wide area outside the fissures for adequate retention. According to Le Bell[25], the fact that the fissures were opened before the sealing might have allowed a plug of resin to be formed, instead of a thin layer of varying thickness. This plug would enhance the adherence to the etched surface. In addition, this plug might provide more resistance to wearness.[44] The third advantage is the ability to diagnose the extent of the carious lesion more accurately. Thus when needed, it is possible to switch the treatment procedures from sealant to preventive resin

restoration. The fouth advantage would be that the risk of microleakage can also be reduced when the fissure is enlarged.[11]

Air abrasion technique, introduced in the 1950s,[4] also has been suggested for the preparation of occlusal surface before sealant application.[42,43] Air abrasion system uses a high-velocity stream of purified aluminum oxide particles propelled by air pressure onto the cleaned and dried tooth surface, revealing areas of enamel decalcification. Stains and the organic plugs found in most pits and fissures could be removed, revealing carious extensions into the subsurface areas of enamel.[6] The abrasive action cleans and widens the pits and fissures.[16] Longer and repeated exposure can excavate incipient caries, preparing the tooth surface for the placement of bonded resin materials.

In this study, the percentage of fissure penetration in acid etching only groups (group 1 and 2) was significantly low ($P<0.05$) than that of other methods. Air abrasion groups and bur preparation groups showed similar fissure penetration percentage although bur preparation groups showed slightly higher measures. There was no statistically significant difference between filled and unfilled sealants, although unfilled sealant showed slightly higher penetration percentage in all three preparation methods.

In this study, acid etching only groups showed significantly more microleakage ($p<0.05$) than bur preparation groups

regardless of filled or unfilled sealants. There were no significant differences between acid etching only groups and air abrasion groups. When air abrasion reappeared on the market in the 1990s, it was claimed that it could prepare enamel surfaces for pit and fissure sealants in a manner similar to acid etching.[16] Hatibovic-Kofman *et al.*[19] reported that air abrasion without acid etching was similar to acid etching alone but inferior to bur preparations in its effect on microleakage. In this study, air abrasion method was combined with acid etching hoping to show significant difference over acid etching alone method. Although statistically no significance was found, superior result was gained with air abrasion method. Microleakage score 0 and 1, meaning that dye penetration did not exceed 10% in depth(Figure 2.1.~ 2.4.), may be considered to be clinically successful, and in this study 21 teeth (88%) in the group with bur preparation with filled sealants were included in this category. In same category, acid etching only with filled sealant group showed lowest clinical success(67%). In acid etching and air abrasion groups, filled sealants showed more microleakage than unfilled sealants, although statistically no difference was indicated. However, in bur preparation groups, filled sealant showed less microleakage (teeth out of score 0 and 1 were 18, which was 88%). Traditionally, it was suggested that less viscous unfilled sealant might have

greater ability in penetration and adaptation resulting in less microleakage.[10,19] Stephan *et al*. found superior penetration into deep fissures with low viscosity sealants.[35] Rock *et al*. reported the retention rate of filled sealants to be less than unfilled sealants.[30] Recent studies by Xalabarde *et al*.[44] and Kim *et al*.[48], however, found no difference in penetration or adaptation between filled and unfilled sealants. In addition, Koch *et al*. found no significant difference in retention rates between the filled and unfilled sealants.

The diversity of methods used to study the microleakage of sealant may explain the inconsistency in reported results.[41] Even within the category of dye penetration studies, there is no standard dye since both methylene blue and basic fuchsin have been widely used.[11,19,37,41,45] In this study, 5% methylene blue was used because the penetration of a dye, although not an absolute measure, can indicate the lack of a perfect seal. Studies on microleakage also vary in their use of thermocycling. There were recent reports that there is no significant difference in microleakage between thermocycled and non-thermocycled groups.[45] In addition, no differences in penetration capabilities or adaptation were detected between these two groups.[44] One study on microleakage did, however, find that degree of microleakage was greater in thermocycled samples.[40] Clearly, the establishment

of a thorough understanding of pit and fissure sealant microleakage depends on the use of more uniform methods.

The sealants chosen in this study were Ultraseal XT *plus* as a filled sealant and Teethmate F-1 as an unfilled sealant. According to Bayne *et al.*,[3] Ultraseal XT *plus* contains 60% of filler by weight which is 1.0~1.5㎛ in diameter and still has enhanced flow compared to other filled resin sealants. When fillers are added, polymerization shrinkage and thermal coefficient decrease and hardness and strength increase. In addition, it has fluoride releasing property. There has been several trials to add the fluoride-releasing property to existing sealants. Although the questions might be arisen regarding the potential decrease of physical properties by adding the fluoride, the recent studies[7,47] showed that there was no statistically significant difference in microleakge between fluoride containing sealant and non-fluoride containing sealants. To avoid controversies, however, both filled and unfilled sealants with fluoride were chosen in this study. The manufacturer of Ultraseal XT *plus* recommends the use of a drying agent after acid etching and prior to sealant application. The drying agent is ethyl alcohol and it is thought to remove any residual moisture left after air drying the acid etched tooth surface. Sealants are generally hydrophobic, and acid etching improves the ability of the sealant to wet the enamel surface.[29]

Penetration of Ultraseal XT *plus* into fissures was reported to be significantly increased when the drying agent was used.[1] Manufacturer explains that Primadry® may further improve the ability of the sealant to wet enamel by complete drying of the acid-etched surface, which allows superior penetration and resin tag formation resulting in reduced microleakage. However, this study failed to show the effectiveness of the drying agent.

On the other hand, Teethmate F-1® is unfilled Bis-GMA sealant mainly comprised of dimethacylate and also releases fluoride. According to Kozai *et al*., Teethmate F-1® has greater contact angle than Ultraseal XT *plus* which means it has less wetability to enamel surface.[24]

The present study demonstrated and confirmed that enlarging the fissures using the bur provided more surface area to retain the sealant and a thicker layer of sealant rather than the thin layer resulting after conventional sealant application (acid etch only). Because an increased surface area will enhance the retention of sealant, the enameloplasty technique should be considered whenever possible. And although microleakage score was not significantly lower in air abrasion group, fissure penetration percentage was similar with bur preparation group and consequently air abrasion can also be used to achieve the superior penetration and increased bulk of sealant. A comparison between

the two surface treatment techniques regarding shear bond strength and clinical longevity of sealant should be followed to make microleakage study more reliable in the future. In addition, as the filled sealant has superior physical property over the unfilled sealant but no significant difference was found in this study, more studies regarding both types of sealants must be established.

Ⅴ. CONCLUSION

This study was performed to compare fissure penetration and microleakage of filled and unfilled sealants after different occlusal surface treatment methods. Acid etching only, bur preparation and air abrasion groups were each sealed with filled and unfilled sealants and the following results were obtained.

1. Superior results in both sealant penetration and microleakage were shown when the tooth surface was prepared by a bur (group 3 & 4). ($P<0.05$)
2. The sealant penetration rate was significantly higher in air abrasion groups (group 5 & 6) than acid etching groups (group 1 & 2). ($P<0.05$)
3. Acid etching only groups (group 1 & 2) and air abrasion groups (group 5 & 6) yielded similar results in microleakage. ($P>0.05$)
4. No significance was found between filled and unfilled sealants.

According to this study, before sealant application, the bur preparation is recommended whenever possible. However, further comparison studies on bur preparation and air abrasion technique should follow.

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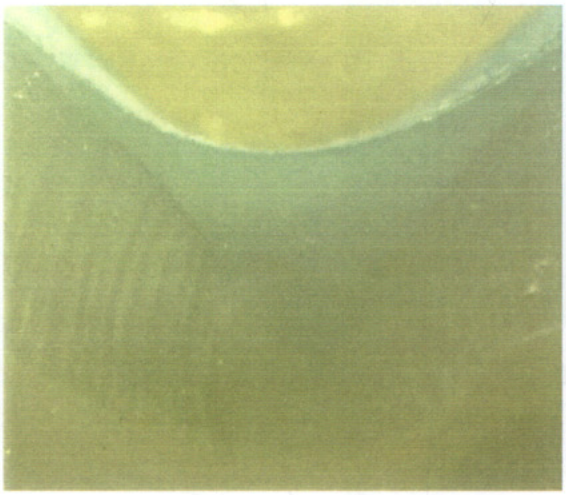
Figures



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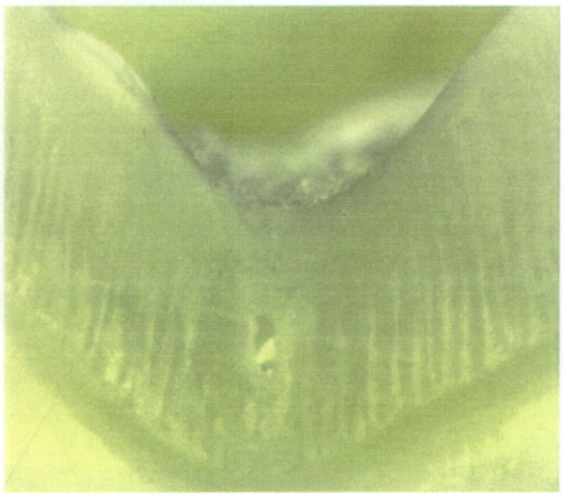

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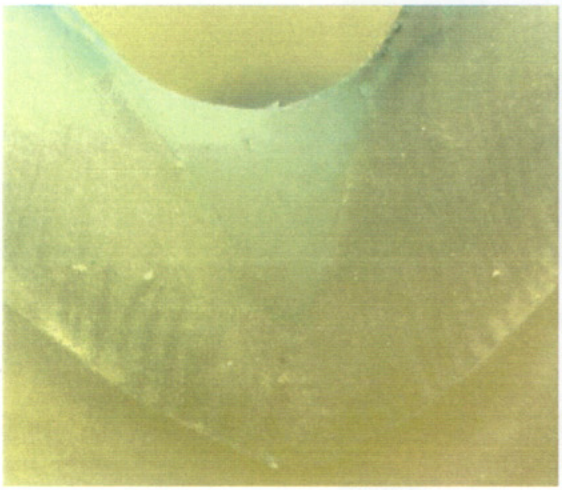

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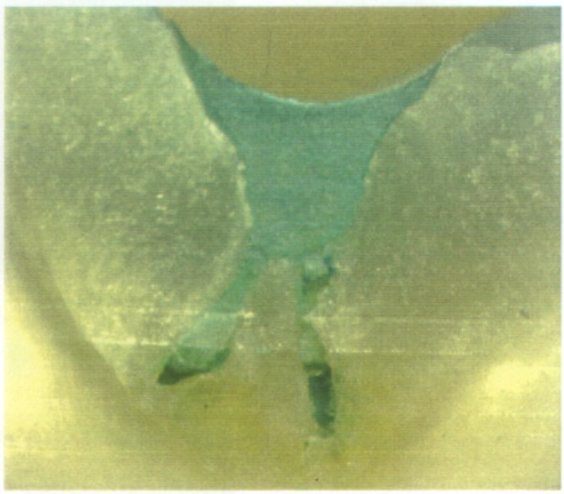

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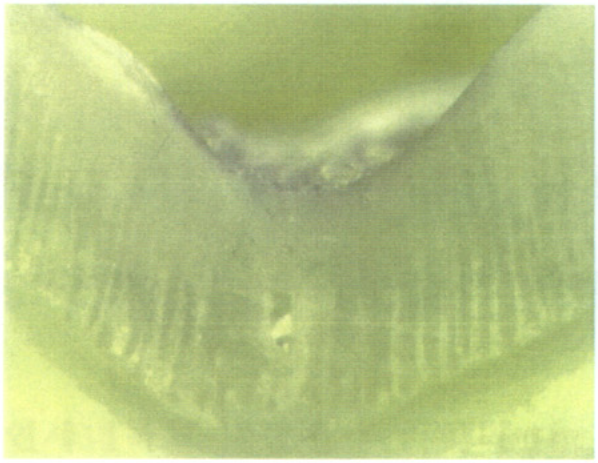

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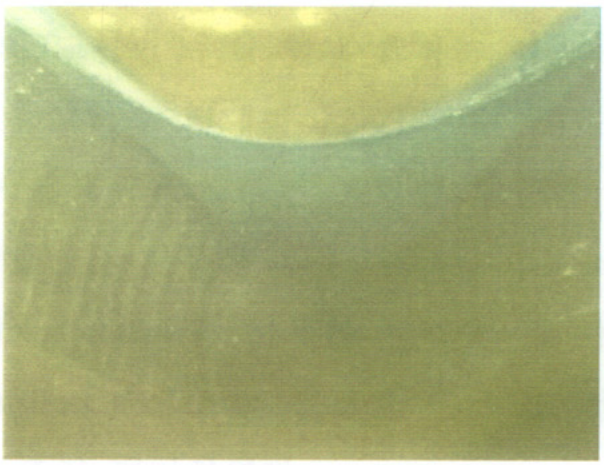

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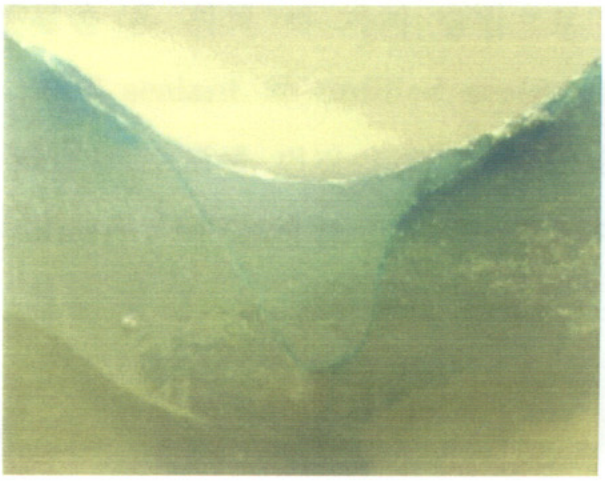

Figure 3-3.

국문 요약

치면 열구 처리방법에 따른 전색제의 열구 침투도 및 미세누출에 관한 연구

연세대학교 대학원 치의학과

정 지 영

치면열구 전색제의 교합면 우식 억제 효과는 이미 잘 알려져 있는 바이다. 이런 전색제의 유지력을 극대화시키기 위한 여러 시도들이 이루어져 왔는데 그중 하나가 전색제의 도포전 교합면 열구의 처리방법이다. 본 실험에서는 산부식 방법만을 사용한 경우, rotary instrument를 이용한 기계적 삭제 방법 및 air abrasion을 사용한 방법등 세 가지 조건으로 나누어 filled sealant 와 unfilled sealant를 도포한 후 이들 전색제의 열구 침투도와 미세누출 정도를 측정 평가하였다.

최근에 교정을 목적으로 발거된 72개의 건전한 소구치를 각각 24개씩 세 군으로 나누어 분배한 후 위의 세 가지 방법으로 처리하였다. 세 군의 치아를 각각 12개씩 filled sealant 와 unfilled sealant로 도포한 후 총 여섯 군의 치아를 5℃와 55℃의 수조에 번갈아 20초씩 1000회의 열순환을 실시하고 5% methylene blue용액에 24시간 보관한 뒤 각각의 치아를 근심 소와와 원심 소와에서 협설 방향으로 절단하여 144개의 절단면을 얻었다. 이렇게 얻어진 시편을 입체 현미경 (Olympus, Japan)을 통하여 영상을 컴퓨터에 입력한 후 화상 분석 프로그램 (Image-Pro plus ver 3.0)을 이용하여 전색제 및 색소의 침투 깊이를 측정하고 비율을 계산하여 전색제의 열구침투도와 미세누출 정도를 평가하여 다음과 같은 결과를 얻었다.

1. bur를 이용하여 기계적 삭제를 시행한 군에서 통계학적으로 유의성 있게 치면열구 전색제의 열구침투도가 증가하였고 미세누출은 감소하였다. ($P<0.05$)

2. air abrasion방법으로 처리한 군이 산부식 방법만을 사용한 군보다 통계학적으로 유의성 있게 치면열구 전색제의 열구침투도가 높았다. ($P<0.05$)

3. 산부식 방법만을 사용한 군과 air abrasion방법으로 처리한 군 사이에서 미세누출은 유의 차가 없었다. ($P>0.05$)

4. 세 가지 방법으로 처리한 후 filled sealant 와 unfilled sealant를 도포한 경우 열구침투도와 미세누출은 통계학적인 유의 차가 없었다. ($P>0.05$)

핵심 되는 말 : 치면열구 처리 방법, 치면열구 전색제, 열구 침투도, 미세누출