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# *In Vitro* Evaluation of Microleakage and Penetration of Hydrophilic Sealants Applied on Dry and Moist Enamel

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**Abstract**

This study aimed to evaluate the microleakage and penetration of two hydrophilic sealants, Embrace Wetbond™ and Ultraseal XT® hydro™, when applied on dry and moist enamel, as compared to a conventionally used hydrophobic sealant, Clinpro™.

A total of 60 sound human third molars were randomly divided into 5 groups according to the enamel moisture control and the sealant material used. After sealant application, the teeth were thermocycled and immersed in 1% methylene blue dye. Subsequently, the teeth were sectioned twice and the sections were examined using an optical microscope and image analysis software.

Application of Embrace Wetbond™ on either dry or moist enamel resulted in more microleakage than Clinpro™. Application of Ultraseal XT® hydro™ on dry enamel showed a similar level of microleakage to Clinpro™, but application on moist enamel resulted in more microleakage. There were no significant differences between the groups in penetration.

In conclusion, application of hydrophilic sealants on moist enamel did not improve the sealing ability and showed lower sealing ability than that of Clinpro™ applied on dry enamel.

**Key words :** Hydrophilic sealant, Microleakage, Penetration

## Ⅰ. Introduction

Pit and fissure sealants are commonly used to prevent occlusal caries[1,2]. Previous studies have reported that single application of resin sealants reduced caries by 4 - 54% while repeated application reduced caries by 69 - 93%[3-6].

Resin-based sealants and glass ionomer cements are available as sealant materials, and resin-based sealants are preferred for their higher retention rate[7]. However, clinicians often experience difficulties in moisture control when applying resin-based sealants to teeth in uncooperative pediatric patients or to partially erupted teeth.

The wet-bonding technique has been used in dentin bonding to prevent the collapse of the collagen network and simultaneously promote penetration of resin monomers to achieve mechanical interlocking[8]. However, it is difficult to fabricate moist dentin and dry enamel simultaneously in a clinical setting, which has encouraged further research into bonding to moist enamel[9]. With advances in adhesive dentistry, many dentin bonding agents containing hydrophilic monomers have

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shown satisfactory bond strength to moist enamel[9,10].

Embrace Wetbond™ (Pulpdent, Watertown, MA, USA) is a moisture-activated hydrophilic resin sealant. It contains di-, tri-, and multi-functional acidic monomers in a proprietary formula[11]. Ultraseal XT® hydro™ (Ultradent, Soth Jordan, UT, USA) is another hydrophilic resin sealant with unique hydrophilic chemistry[12]. The manufacturers recommend that both the hydrophilic products should be applied on moist enamel. Previous studies evaluating the performance of Embrace Wetbond™ have reported controversial results[13,14], and only a few studies have evaluated the performance of the recently developed Ultraseal XT® hydro™[15,16].

The sealing ability of pit and fissure sealant is one of the key factors in caries prevention. A poor sealing ability causes microleakage, which could lead to caries developing below the sealant material. Long-term retention of the sealant is also an important factor in caries prevention and is dependent on the ability of the sealant to penetrate into fissure[17]. The objective of this study was to assess the microleakage and penetration of two hydrophilic sealants when applied on dry and moist enamel.

## Ⅱ. Materials and methods

### 1. Materials

A total of 60 extracted sound, caries-free human third molars were used in this study. After removing the attached tissues, the teeth were stored in sterile distilled water at 4℃ until use. Embrace Wetbond™ and Ultraseal XT® hydro™, hydrophilic sealants, were used as the experimental group and Clinpro™ (3M ESPE, St. Paul, MN, USA), hydrophobic sealant, was used as the control group (Table 1). This study was approved by Wonkwang Institutional Review Board (IRB File No.: WKIRB-201509-BR-005).

### 2. Methods

#### 1) Sealant application

The teeth were randomly divided into 5 groups consisting of 12 teeth each (Table 2). After cleaning the occlusal surfaces using a brush with fluoride-free pumice in a low-speed handpiece, debris remaining in the pits and fissures was removed using an explorer. The occlusal surfaces were etched with 35%

**Table 2.** Description of the experimental groups

| Group | N* (sections) | Sealant | Enamel Surface |
|---|---|---|---|
| CD (control) | 48 | Clinpro™ | Dry |
| ED | 48 | Embrace Wetbond™ | Dry |
| EM | 48 | Embrace Wetbond™ | Moist |
| UD | 48 | Ultraseal XT® hydro™ | Dry |
| UM | 48 | Ultraseal XT® hydro™ | Moist |

* 12 teeth were assigned to each group and each tooth provided 4 sections
CD = Clinpro™ on dry enamel, ED = Embrace Wetbond™ on dry enamel, EM = Embrace Wetbond™ on moist enamel, UD = Ultraseal XT® hydro™ on dry enamel, UM = Ultraseal XT® hydro™ on moist enamel.

**Table 1.** Type and composition of resin sealants in this study

| Brand Name | Type | Composition | Manufacturer |
|---|---|---|---|
| Clinpro™ | Hydrophobic | Matrix: Bis-GMA, TEGDMA, EDMAB, hydroquinone, silica, TBA TFB, $TiO_2$, rose Bengal sodium<br>Fluoride : Yes | 3M ESPE |
| Embrace Wetbond™ | Hydrophilic | Matrix: UEDMA, BMEP, HEMA, TMPTMA, $H_2O$, catalysts<br>Fillers: $SiO_2$, NaF(37wt%)<br>Fluoride: Yes | Pulpdent |
| Ultraseal XT® hydro™ | Hydrophilic | Matrix: TEGDMA, DUDMA, Methacrylic acid<br>Filler : Mixture of inorganic fillers(53wt%)<br>Fluoride : Yes | Ultradent |

Bis-GMA = bisphenol A glycidyl dimethacrylate, TEGDMA = triethylene glycol dimethacrylate, EDMAB = ethyl 4-dimethyl aminobenzoate, TBA TFB = tetrabutylammonium tetrafluoroborate, UEDMA = aliphatic diurethane dimethacrylate, BMEP = bis-methacryloyl ethyl phosphate, HEMA = 2-hydroxyethyl methacrylate, TMPTMA = trimethyloyl propane trimethacrylate, DUDMA = diurethane dimethacrylate.

phosphoric acid (Ultra-Etch®, Ultradent, USA) for 20 seconds and rinsed. Dry enamel was prepared by drying the tooth with oil-free air for 10 seconds until a frosty white appearance was achieved. Moist enamel was prepared by lightly drying the tooth with oil-free air for 1 second and removing excessive moisture with a cotton pellet to achieve a shiny appearance.

After sealant application, there was a waiting period of 20 seconds to allow the sealant to penetrate sufficiently into the pits and fissures. The teeth were then light-cured using an LED light-curing unit (VALO®, Ultradent, USA) at an intensity of 1,000 $mW/cm^2$ for 20 seconds.

2) Sample preparation

The sealed teeth were stored in distilled water at 37℃ for 24 hours and thermocycled for 1,000 cycles between 5℃ and 55℃, with a dwell time of 30 seconds in each bath. After removal of the remaining moisture using a gauze, the apices were sealed with a resin-modified glass ionomer (Ionoseal®, VOCO, Hamburg, Germany) and the tooth surface was coated with two layers of nail varnish except for 1 mm around the sealant area. All teeth were immersed in 1% methylene blue solution (Daejung Chemical Co., Shiheung, Korea) for 24 hours and then rinsed with water. After embedding the teeth in transparent orthodontic resin (Orthoplast, Vertex Dental, Soesteberg, Netherlands), the teeth were sectioned twice longitudinally in a buccolingual direction using a low-speed diamond saw (Isomet, Buehler, Minneapolis, MS, USA), obtaining four sections per tooth.

3) Evaluation of microleakage and penetration

Each sample was observed with an optical microscope (Axioplan, Zeiss, Oberkochen, Germany) at ×50 magnification and was photographed using a digital camera. The images were analyzed using image analysis software (Toupview 3.7, Touptek, Hangzhou, China). Microleakage and penetration were evaluated as described by Celiberti and Lussi[18]. The ratio of microleakage was evaluated based on the proportion of the length of the dye penetration to the length of the total tooth-sealant interface (Fig. 1). The rate of sealant penetration into the fissure was evaluated based on the proportion of the area unfilled by the sealant to the total fissure area (Fig. 2).

4) Statistical analysis

SPSS 18.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The Kruskal-Wallis test was used to determine the significance of differences between experimental groups, with

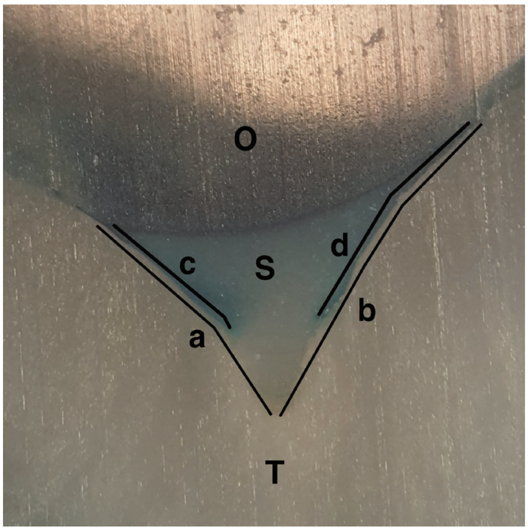


**Fig. 1.** Illustration of measurement of the microleakage proportion (T: tooth, S: sealant, O: orthodontic resin). (a) and (b) indicate the length of the total tooth-sealant interface measured in pixels using the image analysis software. (c) and (d) indicate the length of the dye penetration measured in pixels. The microleakage proportion = (c + d) / (a + b).

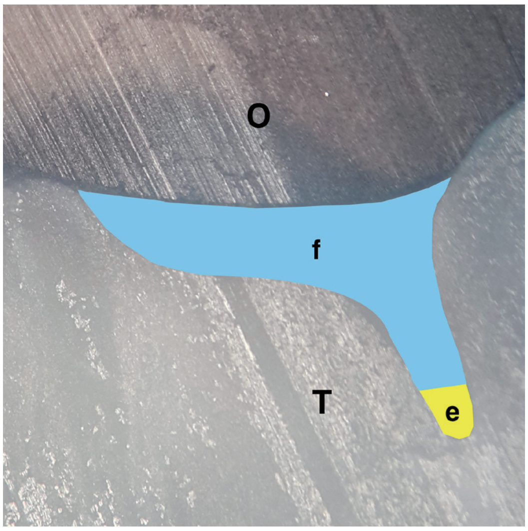


**Fig. 2.** Illustration of measurement of the unfilled area proportion (T: tooth, O: orthodontic resin). (e) indicates the unfilled area measured in square pixels and (f) indicates the total fissure area, except for the unfilled area, measured in square pixels using the image analysis software. The unfilled area proportion = e / (e + f).

a 95% confidence interval. The significance of the results between each group was verified using the Mann-Whitney U test ($p < 0.005$, Bonferroni correction).

## Ⅲ. Results

### 1. Microleakage

The means and standard deviations of the microleakage proportions are shown in Table 3. Group UD showed microleakage similar to that of group CD ($p > 0.005$, Mann-Whitney U test using Bonferroni correction), while group UM showed significantly more microleakage than group CD ($p < 0.005$). Both group ED and group EM showed significantly more microleakage than group CD ($p < 0.005$). There were no significant differences among groups UM, ED, and EM ($p > 0.005$).

### 2. Penetration

The means and standard deviations of the proportions of unfilled area are shown in Table 4. Groups CD and UD showed slightly higher penetration than the other groups, but the differences were not statistically significant ($p > 0.05$, Kruskal-Wallis test).

## Ⅳ. Discussion

Previous clinical trials that compared the retention rates of Embrace Wetbond™ and hydrophobic sealants have reported controversial results. Several studies reported that Embrace Wetbond™ had similar or higher retention rates than hydrophobic sealants. Accordingly, it was suggested that Embrace Wetbond™ is particularly appropriate for use in public dental health programs because of the ease of application and its reduced sensitivity to moisture[19,20]. In contrast, Schlueter *et al.*[21] reported a lower retention rate of Embrace Wetbond™, and suggested two failure factors. First, it was difficult to fabricate the slightly moist enamel as recommended by the manufacturer. Second, it was possible that physical properties were undermined due to a low cross-linking density of the hydrophilic sealant.

The sealing ability of a sealant material can be evaluated using a microleakage test. In this study, only Ultraseal XT® hydro™ applied on dry enamel showed microleakage similar to that of Clinpro™. Embrace Wetbond™ applied on both dry and moist enamel and Ultraseal XT® hydro™ applied on moist enamel showed significantly more microleakage than Clinpro™. Previous studies have reported that hydrophilic sealants showed more microleakage applied on moist enamel than on dry enamel[22,23].

There are two explanations for the improved sealing ability of hydrophilic sealants on dry enamel as compared to moist enamel. The first is the failure of wet-bonding of the hydrophilic sealant to moist enamel. Moisture present on etched enamel plugs the microporous surface, which impedes the formation of resin tags and ultimately weakens the bond[24]. Acid-etched enamel has high surface energy and creates a strong bond to moisture. Thus, for effective bonding to moist enamel, the moisture should be displaced or combined with a

**Table 3.** Microleakage proportions in each group

| Group (N=48) | Mean ± SD (%) |
|---|---|
| CD (control) | 15.53 ± 10.58[a] |
| ED | 29.19 ± 21.07[b] |
| EM | 32.35 ± 25.11[b] |
| UD | 13.26 ± 12.82[a] |
| UM | 30.38 ± 23.17[b] |

Kruskal-Wallis test followed by Mann-Whitney *post-hoc* analysis.
a,b : Mann-Whitney grouping, which means that values with the same letters are not significantly different, $p > 0.005$ (Bonferroni correction).
CD = Clinpro™ on dry enamel, ED = Embrace Wetbond™ on dry enamel, EM = Embrace Wetbond™ on moist enamel, UD = Ultraseal XT® hydro™ on dry enamel, UM = Ultraseal XT® hydro™ on moist enamel, SD = standard deviation.

**Table 4.** Proportions of unfilled area in each group

| Group (N=48) | Mean ± SD (%) |
|---|---|
| CD (control) | 2.01 ± 4.09 |
| ED | 5.15 ± 8.36 |
| EM | 5.87 ± 10.27 |
| UD | 2.66 ± 6.18 |
| UM | 4.59 ± 9.63 |

Kruskal-Wallis test.
No significant difference between the groups ($p > 0.05$).
CD = Clinpro™ on dry enamel, ED = Embrace Wetbond™ on dry enamel, EM = Embrace Wetbond™ on moist enamel, UD = Ultraseal XT® hydro™ on dry enamel, UM = Ultraseal XT® hydro™ on moist enamel, SD = standard deviation.

bonding agent[9]. Previous studies have reported that bonding agents containing ethanol or acetone solvents, which enhance displacement of moisture, in addition to hydrophilic monomers, showed satisfactory bond strength to moist enamel[25,26]. The two hydrophilic sealants used in this study had hydrophilic compositions, but they lacked such solvents[14,16]. This could leave excessive moisture on the bonding interface, which in turn could interfere with the bonding and polymerization of the resin[10].

The second is water sorption by hydrophilic monomers. Water sorption increases with the hydrophilicity of monomers[27]. The absorbed moisture undermines bonding durability by accelerating the hydrolytic degradation of the bonding interface[28]. It may also induce structural defects in the resin, which further accelerates moisture sorption and generates internal swelling stress[29]. In this study, the teeth were kept in distilled water for 24 hours after sealant application and were thermocycled in water, which implies that water sorption by the hydrophilic monomers may played a role in the increasing microleakage.

It was also found that Embrace Wetbond™ applied on dry enamel showed more microleakage than Clinpro™. This may have been due to the moisture-activated nature of Embrace Wetbond™. The manufacturer specifies that the tooth surface should not be desiccated because Embrace Wetbond™ contains acidic monomers and is activated by moisture. Although the exact composition of Embrace Wetbond™ is unknown, another moisture-activated resin adhesive (Smartbond, Gestenco International, Gothenburg, Sweden) showed a low bond strength to dry enamel[30].

In terms of the rate of sealant penetration into fissures, there were no significant differences between the groups in this study, which was consistent with the findings of Khogli *et al*.[22]. Alternatively, some studies have reported that hydrophilic sealant showed less penetration in moist enamel than in dry enamel[14,23]. Beslot-Neveu *et al*.[23] stated that hydrophilic sealant could not penetrate to the bottom of the fissures while displacing water because its surface energy is lower than that of water. Eliades *et al*.[14] suggested that residual moisture impeded the penetration of the sealant by forming a liquid meniscus due to surface tension at the bottom of the fissure.

The viscosity of the sealant material also affects the penetration of the sealant into fissures. Sealants with low viscosity showed higher penetration than sealants with high viscosity[31]. In this study, the viscosities of sealant materials were not assessed. However, a previous study reported that Embrace Wetbond™ has lower viscosity than hydrophobic sealants[13]. Therefore, the relatively lower penetration of Embrace Wetbond™ observed in this study may not be due to its viscosity.

The use of pit and fissure sealant is important in caries prevention method for pediatric patients who lack the ability to manage their oral hygiene appropriately. Thus, many efforts have been made to develop moisture-insensitive sealant materials that bind strongly to teeth. Based on our findings, the current hydrophilic sealants may be insufficient for achieving this purpose.

One of the limitations of the present study was that fissure morphology, which is related to the penetration ability of sealants, was not considered. In addition, the collected teeth were examined visually in the present study, and use of adjunctive methods such as laser fluorescence may improve the accuracy of caries detection.

## Ⅴ. Conclusions

This study evaluated the microleakage and penetration of two hydrophilic sealants applied on dry and moist enamel. Embrace Wetbond™ showed more microleakage than Clinpro™ when applied on both dry and moist enamel. Ultraseal XT® hydro™ showed microleakage similar to that of Clinpro™ when applied on dry enamel, but showed more microleakage on moist enamel. There were no significant differences in penetration between the groups. In conclusion, application of hydrophilic sealants on moist enamel did not improve the sealing ability and showed reduced sealing ability compared to Clinpro™ applied on dry enamel.

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국문초록

# 법랑질 수분 처리에 따른 친수성 치면열구전색제의 미세누출과 열구 침투도 평가

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이 연구의 목적은 2종의 친수성 치면열구전색제를 건조한 법랑질과 습한 법랑질에 적용하였을 때의 미세누출과 열구 침투도를 평가하는 것으로, 기존의 소수성 전색제인 Clinpro™와 비교하였다.

건전하고 우식이 없는 제3대구치 60개를 법랑질 수분 처리 방법과 전색제 종류에 따라 12개씩 다섯 군으로 나누었다. 전색제 적용 후 치아들을 열순환 시킨 뒤 1% 메틸렌블루 용액에 담갔다. 그리고 치아들을 두 번씩 절단한 뒤 절단면들을 광학 현미경과 이미지 분석 프로그램을 이용해 관찰하였다.

Embrace Wetbond™는 건조한 표면과 습한 표면 모두에서 Clinpro™보다 많은 미세누출을 나타냈다. Ultraseal XT® hydro™는 건조한 표면에서는 Clinpro™와 유사한 미세누출을 보였으나, 습한 표면에서는 많은 미세누출을 보였다. 열구 침투도는 모든 군에서 통계적으로 유의한 차이가 없었다.

결론적으로 친수성 전색제들을 습한 법랑질에 적용했을 때 변연 봉쇄성이 향상되지 않았으며, 건조한 표면에 적용한 Clinpro™보다 낮은 변연 봉쇄성이 관찰되었다.

**주요어:** 친수성 치면열구전색제, 미세누출, 열구 침투도