ว ทันต จุฬาฯ 2558;38:221-228
CU Dent J. 2015;38:221-228

Original Article
บทวิทยาการ

Fluid Emerging from Etched and Unetched Dentin Surface under Carious Lesions in Primary Mandibular Second Molars

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Abstract

Background: Only a limited number of reports existed about fluid flow through dentin in primary teeth, none of them were studied in carious dentin *in vivo.*

Objectives: To study the dentinal fluid flow on both etched and unetched dentin surfaces beneath carious lesions of vital primary mandibular and second molars by using impression and replica technique.

Materials and Methods: Thirty primary lower second molars were included in this study. They were divided into 3 groups according to the depth of the carious lesions. The teeth were anesthetized with 3% plain mepivacaine. Caries was removed with standard procedure. A silicone impression material was then used to record the floor of the cavities before and after acid etching. Replicas were made by casting the impressions with epoxy resin and examined under scanning electron microscope.

Results: Fluid droplets, round and ovoid in shape, were discovered on the replica of un-etched dentin surfaces while porous surfaces were shown on etched dentin surfaces. One-way ANOVA statistical analysis showed significant differences in the diameters of dentinal tubules, the numbers of dentinal tubules/mm^2 among the 3 different cavity depths in the etched groups, the diameter of fluid droplets and the numbers of fluid droplets among the 3 cavity depths in the un-etched groups.

Conclusions: Fluid droplets were found on the replicas of un-etched dentin surfaces while the openings of dentinal tubules were found on the replicas of etched dentin surfaces. Both fluid droplets and dentinal tubules increased in diameters when cavity depth increased.

(CU Dent J. 2015;38:221-228)

Key words: *carious dentin; dentin depth; dentinal fluid; fluid droplet; primary teeth; replica technique*

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Introduction

Dentin becomes permeable after enamel or cementum is lost. Bacteria can penetrate and diffuse its toxin through the exposed dentinal surface into the dental pulp (Mjor, 2009). However, spontaneous outward flow of dentinal fluid helps prevent the invasion of bacteria and its toxin with a simple diffusion of chemicals such as Evan's blue dye (Vongsavan and Matthews, 1992; Ciucchi et al, 1995). Smeared layers and plugs produced during a cavity preparation can reduce dentinal permeability by occluded dentinal tubule (Pashley, 1985). Therefore removing them by acid etching can increase dentin permeability (Mjor, 2009). There are also other factors that increasing dentin permeability toward the pulp such as the number/unit area and diameter of dentinal tubules (Pashley, 1985).

Fluid flows through dentin can affect the bonded strength of dental adhesive and restoration. Our previous studies suggested that the application of simulated pulpal pressure resulted in a decreased of microtensile bond strength of both total etch and self-etch systems. Fluid flows though dentin may be one of the factors that influence the adhesion of bonding on dentin surface (Saelim, 2012; Akkho, 2014).

Measurement for small volume of fluid flow through dentin required delicate and sophisticate techniques which is only suitable for animal experimentation (Vongsavan and Matthews, 1992). The impression and replica technique, which was first introduced by Bharali et al., 1988 for recording sweat droplets in rats hind paw, might be suitable for the use of recording fluid droplets on the dentin surface as the fluid flow through dentin *in vivo*. This techinque was used to record emerging fluid droplet on exposed permanent dentin (Itthagarun and Tay, 2000; Kerdvongbundit et al, 2004). The same technique was also used to observe fluid droplets on primary incisors after simulated pulpal pressure *in vitro* (Rangcharoen, 2011). However, fluid flow through infected dentin in primary molar remains unknown. Our purpose was to investigate the fluid flow through dentin on both etched and un-etched dentinal surface beneath the carious lesion of vital primary molar by using impression and replica technique.

Material and methods

The protocol was approved by Human experimentation committee, faculty of Dentistry, Chiang Mai University (certificate number 14/2557). The inform consent was signed by the subject's guardians. This study was conducted in pediatric dental clinic at faculty of Dentistry, Chiang Mai University.

Thirty mandibular second primary molars with occlusal caries, not deeper than 2.0 mm from the surface, in healthy and co-operative children, age 5-11 years, were recruited in this study. Bite-wing radiographs were taken to approximate the depth of carious lesions and rule out proximal caries. Teeth were divided into 3 groups by the depths of the cavity:

Group A: the depth of cavity between 0.50-1.00 mm (n=10),

Group B: the depth of cavity between 1.01-1.50 mm (n=10),

Group C: the depth of cavity between 1.51-2.00 mm (n=10).

The selected tooth was anesthetized by an inferior alveolar nerve block with 3% mepivacaine plain (Scandonest®, Septodont). Rubber dam was applied to isolate the tooth. Cavity was prepared by using high speed round diamond bur. Caries was removed by slow speed round steel bur and spoon excavator until the hard dentin surface was detected. All procedures were done with the same dentist to minimize any technical error.

Two impressions of prepared cavity were taken by using silicone impression material (Xantopren® VL plus: Heraeus, Kulzer, Germany) before and after etching procedure. The cavity was mop dried with cotton pellet and leave for 30 seconds before the first silicone impression of un-etched dentin was taken. A mixing impression was loaded into the 1 ml syringe and injected onto the floor of the cavity until it was filled up. After the cast is set, the impression was gently removed with an explorer and stored in a sealed container. The prepared cavity was then etched with 37% phosphoric acid gel (Scotchbond, 3M ESPE, St. Paul, MN, USA) for 15 seconds, then rinse with water spray from a triple syringe for 15 seconds. The second impression was taken by using the same procedures. The experimented cavity was then re-etched and restored with composite resin in accordance to the manufacturer's recommendation.

Priorto casting, the depth of the experimented cavity impression was measured by Vernier caliper to re-confirm the actual depth ofthe cavity. The replica was made by casting impression within an hour using a low viscosity epoxy resin (Wilhelm®; Wilhelm chemical industrial Co., Germany) and left at room temperature for at least 8 hours. It was fixed onto a stub and coat with gold-palladium, and examined under scanning electron microscope (SEM, JOEL® JSM 6610LV, JEOL Ltd., Tokyo, Japan) at Medical Science Research Equipment Center, Chiang Mai University, Chiang Mai, Thailand. The digital images of the replica were taken at 250 X, 800 X, 1,000 X and 3,500 X magnifications for analysis. The size of the fluid emerging from the un-etched dentin surface and the diameter of the opening of tubule were measured by using Image J program version 1.45 (National Institute of Health, US). Moreover, the number of tubule/unit area was counted and calculated manually. Descriptive analysis and one way ANOVA were used to compare data of 3 groups. In addition, the Tukey Multiple Comparison test was employed to differentiate the significant differences between each group ($p<0.05$).

Results

The surface of replica recorded from un-etched dentin showed droplets like structure while those recorded from etched dentin showed porous structure with no fluid droplets like structure was found.

The droplets like structure were found on the surface of un-etched dentin replica samples. They were round or ovoid in shape. The mean ± SD diameter of fluid droplets were 2.31 ± 0.67, 4.14 ± 0.91, and 5.67 ± 1.54 µm of the samples ingroup A, B, and C, respectively. The mean ± SD numbers of fluid droplets/mm^2 were 3,145 ± 145.97, 3,822 ± 217.19, and 4,537 ± 204.21 droplets/mm^2 in the group A, B and C, consecutively (Table 1). There were significant differences in diameter of fluid droplets and numbers of fluid droplets/mm^2 among those groups ($p<0.05$, One-way ANOVA).

Examples of the SEM images of the replica of the un-etched exposed dentin surface from group A, B, and C were illustrated in Figure 1. Droplet-like structures at high magnification are shown in Figure 2.

Records from replica of etched dentin surface showed the opening of dentinal tubules-like structure but not fluid droplets like structure as shown in Figure 3. An individual opening of dentinal tubule had round or ovoid shape while coalescence of two or more dentinal tubules were also found. The mean ± SD diameter of dentinal tubules were 2.26 ± 1.01, 4.13 ± 1.11, and 4.99 ± 1.63 µm of samples ingroup A, B, and C, respectively. The mean ± SD numbers of dentinal tubules/mm^2 were 8,154 ± 764.15, 8,845 ± 1,343.89, and 9,573 ± 1,350.51 tubules/mm^2 in the samples of group A, B and C, consecutively (Table 2). The statistical analysis showed that there were significant differences in diameter of dentinal tubules and numbers of dentinal tubules/mm^2 among those groups ($p<0.05$, One-way ANOVA).

Table 1 The mean values of numbers of fluid droplets and diameter on the unetched exposed dentin surface at different cavity depths

Cavity depths (mm)	Mean ± SD No. of fluid (droplets/mm^2)	Mean ± SD Diameter (μm)
0.50-1.00 (n=10)	3,145 ± 145.97*	2.31 ± 0.67*
1.01-1.50 (n=10)	3,822 ± 217.19**	4.14 ± 0.91**
1.51-2.00 (n=10)	4,537 ± 204.21***	5.67 ± 1.54***

*Statistically significant differences in mean numbers of fluid droplets/mm^2 and diameter (one-way ANOVA test, $p<0.05$).

**Statistically significant differences in diameter of fluid droplets and mean numbers of fluid droplets/mm^2 between group A and B (Tukey test, $p<0.05$).

***Statistically significant differences in diameter of fluid droplets and mean numbers of fluid droplets/mm^2 between group B and C (Tukey test, $p<0.05$).

Table 2 The mean values of numbers of dentinal tubules and diameter on the etched exposed dentin surface at different cavity depths

Cavity depths (mm)	Mean ± SD No. of dentinal (tubules/mm^2)	Mean ± SD Diameter (μm)
0.50-1.00 (n=10)	8,154 ± 764.15*	2.26 ± 1.01*
1.01-1.50 (n=10)	8,845 ± 1,343.89	4.13 ± 1.11**
1.51-2.00 (n=10)	9,573 ± 1,350.51****	4.99 ± 1.63***

*Statistically significant differences in mean numbers of dentinal tubules/mm^2 and diameter (one-way ANOVA test, $p<0.05$).

**Statistically significant differences in diameter of dentinal tubule between group A and B (Tukey test, $p<0.05$).

***Statistically significant differences in diameter of dentinal tubule between group B and C (Tukey test, $p<0.05$).

****Statistically significant differences in mean numbers of dentinal tubules/mm^2 between group A and C (Tukey test, $p<0.05$).

Figure and Figure legends

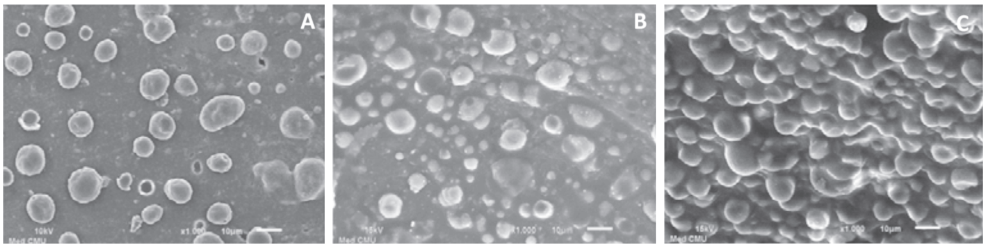

Fig. 1 SEM image showed a replica of the unetched exposed dentin surface between group A, group B, and group C. The image was taken from magnification of x 1000.

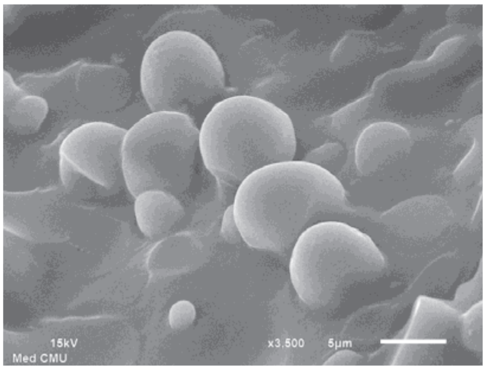

Fig. 2 SEM image showed droplets on a replica of an unetched exposed dentin surface diameter 5.22 μm from group C. The image was taken from magnification of x 3500.

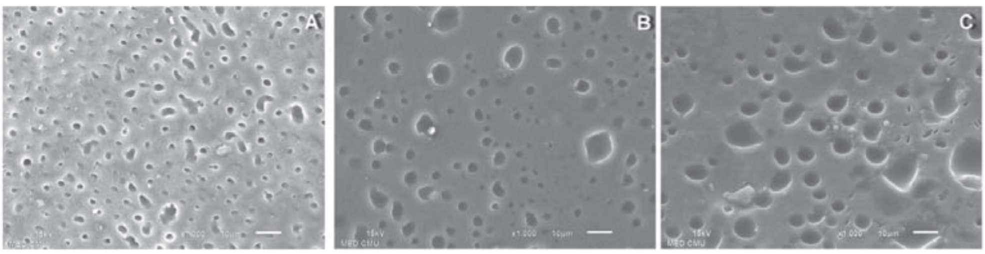

Fig. 3 SEM image showed a replica of the etched exposed dentin surface between group A, group B, and group C. The image was taken from magnification of x 1000.

Discussion

The fluid droplets on replica from un-etched dentin surface in this study confirm the results of the previous *in vitro* study in primary teeth (Rangcharoen, 2011) and in permanent teeth (Kerdvongbundit et al., 2004). The fluid droplets were round or oval in shape. The shapes of fluid droplets observed in this study corresponded to other studies (Rangcharoen, 2011; Chanprasert et al., 2014).

The result from this study supported the study of Koutsi and colleagues (1994) that the permeability increases in depth. The present study was also interested in the permeability of dentin under carious lesion. In dentin under carious lesion, reparative dentin response to caries is rapidly formed, resulting in an irregular tubular pattern (Kinney et al., 2005). Tagami and colleagues (1992) also suggested that sclerosis of dentin beneath a carious lesion obstructed the dentinal tubules and reduced its permeability. These may affect fluid flow rate and the number of fluid droplets/area present on the dentin in this study which showed smaller diameter of fluid droplets and smaller number of droplets/area when compare to our previous studies (Rangcharoen, 2011; Chanprasert et al., 2014). Moreover, during the slow progression of caries period, the mineral can re-precipitated in the intratubular dentin and result in the reduction of dentin permeability (Mjor, 2009). Pashley and colleagues (1991) also reported the lower permeability of carious dentin after excavation and removal of smear layer.

The diameter of dentinal tubule becomes increased in size closer to the dental pulp (Koutsi et al., 1994; Sumikawa et al., 1999). Similarly, the fluid droplets recorded at the deeper dentin (group C) are larger in diameter than those at shallow dentin (group A and B) corresponding to our previous experiment. (Chanprasert et al., 2014)

Furthermore, condensation silicone impression materials that are used to record the fluid droplet from exposed dentin may play a role. These materials consisted of base and accelerator, or catalyst. The condensation reaction produces ethyl alcohol as a by-product disturbed the impression and replica casting processes (Sakaguchi RL, Powers JM, 2012).

Replica of etched dentin surface showed the opening of dentinal tubules without the presence of fluid droplets at all cavity depths. The tubule diameters tend to increase in deeper dentin. This correlated to the results from other studies (Garberoglio and Brannstrom, 1976; Koutsi et al., 1994; Sumikawa et al., 1999). However, our result showed that tubule diameters in each depth of second primary molar teeth are larger than permanent teeth (Garberoglio and Brannstrom, 1976), contrasting Koutsi and colleagues' study, which was reported that tubule diameters measured on buccal dentin in primary molars were smaller than permanent teeth (Koutsi et al., 1994).

Our study found that the mean numbers of dentinal tubules/mm^2 were approximately 8,154, 8,845, and 9,573 tubules/mm^2 in group A, B and C consecutively. The tubule density tends to increase in deeper dentin. This correlated to the results from other study (Koutsi et al., 1994). The density of dentinal tubules found in our study is less than other reports in both primary teeth (Koutsi et al., 1994; Sumikawa et al., 1999) and permanent teeth (Garberoglio and Brannstrom, 1976; Fosse et al., 1992).

The different result of the above studies may be due to factors such as the type and age of the teeth, various distances from dentinoenamel junction, and the small sample size.

Conclusions

Fluid droplets were found on the replica of un-etched dentin surface under carious lesion. They increased in both diameter and number in deeper dentin. Moreover, when compare to previous studies they had smaller diameter and smaller number of droplets/area. No fluid droplet was found on etched dentin surface, instead the openings of dentinal tubules were found on the replicas. The diameter and number/unit area also increase when the depth increase.

Acknowledgements

This study was supported by faculty of Dentistry, Chiang Mai University.

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