## **CHAPTER V**

## DISCUSSION

This study manifested that replica technique can be used to record the fluid droplets and dentinal tubules on exposed dentin of a primary tooth. The replica technique was simple, easy to set up, non-invasively and more convenient to record the dentinal fluid droplets than the techniques employed by Vongsavan and Mathews (1992), Ciucchi and colleagues (1995) and Erturk and Kirzioglu (2007). This technique has advantages that it is less sensitive to thermal effect and pressure changes and not complicated to set up. This method can record the number and size of droplets emerging from dentinal tubules of unetched dentin surface together with hydrated surface of dentinal tubule of etched dentin. However, it has disadvantages that it cannot measure an inward flow and a continuous fluid flow (Kerdvongbundit *et al.*, 2004).

The fluid droplets were discovered on the replicas of cut dentin surfaces in primary teeth after connected to water manometer. However, there was no fluid droplet presented on the dry dentin surface or from the replica of dry dentin surface. These indicated that the droplets come from water in the system flow through the dentinal tubules and emerge on dentin surface.

Sasazaki and Okuda (1995) used the replica technique to study unetched dentin surface of premolar and molar teeth under local anesthesia. They found the exudation from dentinal tubule similar to our study. In contrast, Itthagarun and Tay (2000) use a resin replica technique to study unetched dentin surface of molar teeth. The specimens were anesthetized with 3% Mepivacaine. They found a smear layer, and an absence of dentinal fluid from the unetched dentin surface of the resin replica. This technique was also used to study the sensitive area at cervical root dentin (Absi *et al.*, 1989). They found that the tubular diameter was less than 1  $\mu$ m.

The replicas exhibited fluid droplets from the unetched exposed dentin surface of primary lower incisor when apply intrapulpal pressure to 0, 15, 30 and 45 cmH<sub>2</sub>0 for 30 seconds. The present study's result was similar to that of Kerdvongbundit *et al.* (2004) which were studied in permanent teeth at 30 mmHg. While, we found the fluid droplets from the unetched dentin surface when apply any studied intrapulpal pressure including at the atmospheric pressure (0 cmH<sub>2</sub>O). Kerdvongbundit *et al.* (2004) did not found except at 30 mmHg. Possibly, removing of odontoblasts with sodium hydroxide 3 days before experiment in their study may pay a part of this responsibility. The diameter of fluid droplets in the present study were range from approximately 3-14  $\mu$ m which were agree with Kerdvongbundit *et al.* (2004) (5-10  $\mu$ m).

In addition, the globular shape and some coalesced of fluid droplets were encountered in Kerdvongbundit *et al.* (2004) and our studies.

About half of the unetched exposed dentin samples, the emerging fluid droplets were obtained in both central and peripheral area. The droplets in the central area tended to be larger than those in peripheral area but not statistically significant. On the other hands, the fluid droplets of the replica of an unetched dentin surface were appeared at the peripheral area in all samples. This observation was contradicted to the fact that, in the center, the sizes of dentinal tubules are larger and the lengths of dentinal tubules are shorter (Garberoglio and Brannstrom, 1976; Koutsi *et al.*, 1994;

Sumikawa *et al.*, 1999). Thus, the fluid should easier flow through the dentinal tubules than those from peripheral area. To explain our results, one possible mechanism would be that the smaller dentinal tubules in the peripheral area have the higher capillary force to drive the fluid from the inner tubules than the central area.

In replica of etched dentin surface, we found the opening of dentinal tubules after applied each pressure for 30 seconds. This result corresponded with some study of Kerdvongbundit *et al.* (2004). They found the opening of dentinal tubules in the replica of etched dentin surface during apply 0 mmHg for 5 minutes and during apply 30 mmHg for 30 seconds and 2 minutes. At the longer period (5 minutes) the fluid droplets were appeared during apply 30 mmHg. In the present study, the short period of time (30 seconds) in applying the pressure may be the cause of unseen fluid droplets on etched dentin surface. The duration for applying the pressure may be influence to the flow of fluid droplets on etched dentin surface. Sasazaki and Okuda (1995) reported that there were fluid droplets when the initial setting time was prolonged. The longer initial setting times, the higher quantity of fluid droplets.

The other explanation might be that collagen layers which left over after etching the dentin surface act as absorbents absorbed water into them. The fibrils of dentin matrix would expand after absorb water and other hydrophilic solvents (Pashley *et al.*, 2007).

Moreover, the other possibility was that there seem to have a resistance to the fluid flow through dentin. These resistance might be obtained from these 3 series: (1) debris occluded the dentinal tubule, (2) internal irregularities and mineralized in the dentinal tubule and (3) the presence of odontoblast process in the dentinal tubule (Pashley *et al.*, 1978). However, in this present study, the debris which occluded the

dentinal tubule should be removed by acid etching and thus, the opening of dentinal tubule was then cleared. Sasazaki and Okuda (1995) reported that the etching of dentin was effectively delayed and decreased the exudation of dentinal fluid.

The study of Itthagarun and Tay (2000) had also found the fluid droplets on the etched exposed dentin surface by using the replica technique to study in vital human molars. Their result presented globular fluid droplets on the dentin surface in all replicas, which were contrasted with our study. Their study used the teeth with occlusal caries that may stimulate the forming of tertiary dentin (Mjor, 2009) while we used the intact teeth.

In most cases, the diameter of dentinal tubules on etched dentin surface was unchanged when increasing pulpal pressure, since the size of collagen of dentin matrix increased vertically when the fluid was released from dentinal tubule (Pashley *et al.* 2007). Moreover, the peritubular dentin has highly mineralization and scanty organic matrix (Mjor 2009), therefore only little organic matrix was left for absorbing water after etching.

In present study, the dentin was cut from dentinoenamel junction approximately 1.0 mm. The Mean  $\pm$  SD values of the diameter of dentinal tubule at the central and peripheral area of primary lower central and lateral incisors were 2.77 $\pm$ 0.10 µm and 2.32 $\pm$ 0.09 µm consecutively. The result was different from what Sumikawa *et al.*, (1999) found. They found the diameter of dentinal tubules of human primary upper lateral incisor and canine varied from 1.39 µm to 1.94 µm when removed dentin from dentinoenamel junction at the level of 0.15, 0.80 and 1.45 mm. However, this present study did not further investigate in the rational of these different results.

The diameters of dentinal tubules of dry tooth surface are significant larger than those recorded with replica technique in pressure 0 cmH<sub>2</sub>0. The organic matrix on etched dentin surface was collapse in dry condition. After rehydrated, the dentin matrix expanded since it absorbed water from the system (Pashley *et al.*, 2007).

Garberoglio and Brannstrom (1976) investigated the dentinal tubules of human permanent incisor, premolars and molars teeth at various distance from the pulp using freeze fracture technique. They found that the size of dentinal tubules at the middle of dentin were 1.2  $\mu$ m. Their finding was smaller than those found in the present study because the acid etching was used in our study. It not only removed smear layer but also removed both inorganic and organic substances in the peritubular dentin. Another explanation would be that the dentinal tubule diameter of primary lower incisor is larger than those of permanent teeth. This present finding corresponded to the study of Sumikawa *et al.* (1999). However, Koutsi *et al.* (1994) reported the smaller dentinal tubules diameter of primary molar when compared to those of permanent teeth reported in the studies of Garberoglio and Brannstrom (1976) and Fosse et al. (1992). Different in experimental methodologies may affect to these results.

The tubule diameter of primary lower incisor at the central area of the dentin was statistical significant greater than those at the peripheral area of the dentin surface (P<0.001) similar to the report of Sumikawa *et al.* (1999) who studied the tubule diameters in the primary upper lateral incisor. They also found the diameter of dentinal tubules at the central area were significant larger than those of the distal area (P<0.05). This observation was corresponding to ours.

This present study used Xantopren<sup>®</sup> which was the hydrophobic impression material to record the exposed dentin surface. This material had the mean and standard deviation of water contact angle  $107.9^{\circ}\pm 2.3^{\circ}$  (Boening *et al.*, 1998). It was suitable to take an impression of moist dentin but was not relevant for taking impression in restorative dentistry.

The result from this present study suggested that there was impossible to make the etched dentin dry. As demonstrated in this study, there was fluid filled in the dentin matrix event at 0 cm  $H_2O$  or -30 cm $H_2O$ . Therefore, the hydrophilic bonding material which can penetrate into the hydrated layer of dentin matrix should be considered to obtain the maximum bond strength of the restoration. This study provides the useful information for the dentist to choose the hydrophilic bonding material to restore the primary teeth.