# CHAPTER 2

# REVIEW OF THE LITERATURE

The review of literature in this chapter include Dentin, Dentinal tubules, Dentin permeability, Pulpal pressure, Effect of dentin structure and pulpal pressure to adhesive materials, Dental adhesives and Bond strength test, and Thermocycling.

## 2.1 Dentin

### 2.1.1 Dentinogenesis

In addition to enamel, the other major component of a tooth is dentin. Dentin is a specialized connective tissue that continue forming throughout the life (Linde and Goldberg, 1993). It contains cellular component called odontoblasts which are still viable after tooth eruption, thus dentin has a reparative capacity in response to stimuli. These lead dentin to be different from enamel. However, both dentin and enamel are the same in terms of allowing the permeation of some ions.

Odontoblasts which originate from ectomesenchymal cells of the dental papilla play a role in the formation of dentin known as dentinogenesis (Linde and Goldberg, 1993). Each odontoblast causes a cytoplasmic extension through the thickness of dentin and then initiates odontoblast process that occupies in dentinal tubule.

During dentinogenesis, undifferentiated mesenchymal cells in dental papilla change into odontoblasts by inner enamel epithelium, basement membrane and extracellular matrix (Linde and Goldberg, 1993). Odontoblasts produce the first layer of dentin called mantle dentin in which mineralization takes place. Initial mineralization is in matrix vesicles that are developed from membrane of odontoblasts and deposits at the mineralization front. After mantle dentin is formed, odontoblast forms predentin layer which has no mineralization and separates the mineralized dentin from odontoblasts. Predentin secretes collagen fibers which are removed at the mineralization front and replaced by hydroxyapatite crystals. Thus, predentin keeps in dynamic process and is ready for mineralization in the next layer (Linde and Goldberg, 1993).

**2.1.2 Dentin structure**

Dentin is a hard tissue, consists of 70% inorganic material to form of hydroxyapatite crystals, 10% water and 20% organic substance, of which about 91% is collagen fibers. Most of the collagen is type I and minor component is type V (van Rensburg, 1975). Noncollagenous matrix components include phosphoproteins, proteoglycans, g-carboxyglutamate-n-containing protein (e.g. gla-proteins), acidic glycoproteins, growth factors and lipids. These components make the dentin more elasticity than enamel and cementum. Thus a function of dentin is to support the overlying brittle enamel (van Rensburg, 1975; Pashley, 1985). Dentin is a fluid-filled porous tissue and is formed by odontoblasts which develop from ectomesenchymal cells of the dental papilla. Odontoblasts, the long life span non-dividing cells, produce an

organic matrix for mineralization of dentin (van Rensburg, 1975). They form the dentinal tubules around odontoblastic processes and are slightly tapered, with the wider portion toward the pulp (Pashley, 1985; Rungvechvuttivittaya *et al.*, 1998). They have two functional states that are secretory and resting. Secretory odontoblast is a large plump cell containing large rough endoplasmic reticulum, several golgi apparatus and secretory granules. There are tight junction and gap junction between odontoblastic layers that result in no gap between cells, thus pulpal and dentinal fluid are clearly discriminated. Moreover, gap junction permits ions and molecules including small substances transfer between cells to remain balancing in odontoblasts. Resting odontoblast, a small flattened cell, has a closed nucleus, less cytoplasm and no golgi apparatus. Under an appropriate stimulation, the resting odontoblast acts as secretory role (Goldberg *et al.*, 2011).

Dentin is divided into three types: primary dentin, secondary dentin and tertiary dentin (Yamakoshi, 2009; Goldberg *et al.*, 2011). At first, primary dentin is continuously formed from tooth bud stage until tooth eruption or complete root formation and has the neatly arrangement of dentinal tubules. After tooth eruption, secondary dentin that has slower rate formation than primary dentin is formed and they still has the neatly arrangement of dentinal tubules. Tertiary dentin is responsible to external stimuli (e.g. dentinal caries or fracture exposed dentin) that can lead to traumatized or necrotized odontoblastic cells. This dentin is formed rapidly thus dentinal tubules cannot be arranged neatly. Because of dentin can be formed throughout the life, dentin is thicken and thereafter root canal can be obstructed.

During the initial stages of dentinogenesis, mantle dentin is the first formed dentin and mineralization of dentin matrix commences within the initial increment of mantle dentin (Yamakoshi, 2009; Goldberg *et al.*, 2011). After the layer of mantle dentin has been deposited, circumpulpal dentin which is the major part of dentin is formed. The main part of organic matrix is collagen fibrils which are oriented to the long axis of the dentinal tubules. Human dentin has no blood supply, it is supplied with nutrients and structural materials by blood vessels from the pulp (Pashley, 1985; Rungvechvuttivittaya *et al.*, 1998).

### 2.1.3 Components of dentin

A characteristic of human dentin is the presence of tubules (20-30% of the volume of intact dentin) (Yamakoshi, 2009; Goldberg *et al.*, 2011) which formed around the odontoblastic processes and wider toward the pulp (Thomas, 1985). Dentinal fluid that flows outward and inward within the dentinal tubules is caused by pressure gradient between the pulp and the oral cavity. In addition, rapid flow of fluid through the tubules is thought to be a cause of dentin sensitivity. Also dentinal tubules have reduced the number and diameter according to distance from the pulp, mean number and diameter are about 45,000/$mm^2$ and 2.5 μm at pulpal wall, 19,000/$mm^2$ and 0.8 μm at 3.1-3.5 mm from the pulp (Garberoglio and Brannstrom, 1976).

Peritubular dentin is dentin lining the tubules whereas intertubular dentin inhabit between the tubules. They are different because peritubular dentin has more mineralized (Thomas, 1985), thus it is harder than intertubular dentin. In addition, the

matrix of peritubular dentin consists of fewer collagen fibrils and higher sulfated proteoglycans. Consequently, it can be easily dissolved in acid leading dentin to have more permeable when acid-etching agents are used during dental procedures.

### 2.1.4 Dentin in primary teeth

Dentin-predentin complex consists of cells (e.g. odontoblasts) and extracellular matrix like other connective tissues. Predentin is a part of dentin that seperates odontoblasts from mineralized portion (extracellular matrix). Most characteristic feature of human dentin is the presence of dentinal tubules that influence on permeability of teeth (Thomas, 1985). In general, dentin either in primary or permanent teeth is similar. However, there are some chemical and morphological differences between them. These include the distance from the DEJ to the pulp that affects to dentin thickness, number of dentinal tubules, tubule density, tubular diameter, width of peritubular dentin, and the presence of microcanals (Sumikawa *et al.*, 1999). The presence of microcanals may affect the solid dentin and influence restoration bond strength which makes the restorative treatment more complicated. In addition, other factors such as a small size, a thinness of enamel and rapid spread of the decay may also make the difficulty in restoration. Salama and Tao (1991) found that primary dentin had lower adhesive bond strength than permanent dentin, contrastly, the higher adhesive bond strength of primary dentin was found in Bordin-Aykroyd *et al.* (1992) study. Some studies found no differences in bond strength between them (Fagan *et al.*, 1986; Mazzeo *et al.*, 1995).

## 2.2 Dentinal tubules

Predentin matrix deposited and mineralized around the odontoblast processes to form dentinal tubules (Thomas, 1985). They continue from the DEJ to the pulp and function as a pathway for nutrients to diffuse through dentin (Smith *et al.*, 2012). The shape of tubules is shallow S-shaped apical and nearly straight beneath the incisal edges or cusps. The dentinal tubules divide into few or more terminal branches and unite with other branches of other tubules to form a plexus near DEJ (van Rensburg, 1975). Dentinal tubule decrease its diameter with an increase of tooth age due to the mineralization of peritubular dentin (Mendis and Darling, 1979). The density of dentinal tubules decreases from the crown to the root apex (Carrigan *et al.*, 1984).

### 2.2.1 Dentinal tubules in primary teeth

Dentinal tubule originates from the deposition and mineralization of predentin matrix surrounding odontoblast process that one odontoblast process is one dentinal tubule (Thomas, 1985). Odontoblast process is a highly branched structure which can be anatomosed and performed extensive tubular branching, resulting in great permeability.

In general, there are several contents in dentinal tubule including peritubular dentin, odontoblast process, collagen, nerve fibers and dentinal fluid. Peritubular dentin lines the wall of tubules and has more mineralization than intertubular dentin but less in organic matrix. The tubules diameter is diminished by age, attrition and dental caries. Odontoblast process or Tome's fiber, which is seperated from tubular

wall by periodontoblastic space, extends through the thickness of dentin and is limited to the pulpal third of the tissue. Furthermore, the lamina limitans is the structure like glycosaminoglycan (GAGS) inhibits mineralization and leads the tubule to maintain permeability. Collagen which appears both in periodontoblastic space and peripheral tubules can also play a role in permeability of dentin. A content in dentinal tubules is nerve fibers which are confined to specific area of dentin and do not penetrate more than 100-150 μm. Finally, dentinal fluid contains protein as in plasma and capillary transudate which differs from interstitial fluid. Movement of dentinal fluid also forms the hypothesis of dentin sensitivity (Thomas, 1985).

As previously mentioned, dentin in primary teeth has structural differences compared to permanent dentin. These differences include thinner thickness which results from the short distance from DEJ to the pulp, tubule density and tubular diameter including peritubular width. Tubular density and tubular diameter were increased when closed to the pulp. They both can be found more in primary teeth (Sumikawa *et al.*, 1999). However, another study found that primary molars have less and smaller tubules compared to premolars (Koutsi *et al.*, 1994). Moreover, peritubular dentin was narrowed when closed to the pulp due to the increase deposition of dentin without exact dentinal tubule distribution (Fosse *et al.*, 1992; Sumikawa *et al.*, 1999). In addition, Sumikava *et al*. (1999) found the microcanals or giant dentin tubules in primary incisor teeth.

### 2.2.2 Dentinal tubules in permanent teeth

Garberoglio an Brannstrom (1976) reported the number of tubules and tubule diameter of human permanent teeth as show in Table 1. The number of tubules is widely variant, possible due to distance measurements error and the variation between individual teeth. Dentinal tubules are about 10% by dentin volume, with the higher percentages in the dentin nearby the pulp (Garberoglio and Brannstrom, 1976).

**Table 1** shows the number of dentinal tubules and tubule diameter of permanent tooth at various distance from the pulp (Garberoglio and Brannstrom, 1976).

| Distance from the pulp (mm) | Number of tubules ($1,000/mm^2$) | | Tubule diameter (μm) | |
|---|---|---|---|---|
| | mean | range | mean | range |
| Pulpal wall | 45 | 30-52 | 2.5 | 2.0-3.2 |
| 0.1-0.5 | 43 | 22-59 | 1.9 | 1.0-2.3 |
| 0.6-1.0 | 38 | 16-47 | 1.6 | 1.0-1.6 |
| 1.1-1.5 | 38 | 21-47 | 1.2 | 0.9-1.5 |
| 1.6-2.0 | 30 | 12-47 | 1.1 | 0.8-1.6 |
| 2.1-2.5 | 23 | 11-36 | 0.9 | 0.6-1.3 |
| 2.6-3.0 | 20 | 7-40 | 0.8 | 0.5-1.4 |
| 3.1-3.5 | 19 | 10-25 | 0.8 | 0.5-1.2 |

The tubule densities of premolar were reported by Fosse and colleague (1992). Teeth were taken from patients aged 10-14 years, and cut axiobuccolingually and slightly lateral to the longitudinal axis to the depth of 0.3 mm from the pulpal wall. The average value was 51,368 tubules/$mm^2$. Pashley (1986) reported the numbers of tubules that are approximate 59,000 to 76,000 tubules/$mm^2$ at the pulpal surface of cervical parts of young premolar and molar teeth and are approximate reduction to half near the enamel. The dentinal tubules diameter is approximate 2.5 μm near the pulp and 900 nm near the DEJ (Pashley, 1986).

**2.3 Dentin permeability**

As mentioned earlier, dentin is a structure which is variable and location dependent. Although primary dentin and permanent dentin are similar, they still differ in histology and microstructure. However, the specific characteristic of human dentin in both dentitions is dentinal tubules which are the tubular structure, makes the dentin porous and provides channels for the passage of fluid across dentin. These can affect dentinal fluid movement or dentin permeability and lead to dentin sensitivity of exposed dentin (Pashley, 1986).

Dentin sensitivity is caused by vary stimuli (heat, cold, drying, mechanical, hydrostatic and osmotic pressures) (Michelich *et al.*, 1978). A direct stimulation of neural receptors in the dentinal tubules and an indirect stimulation of receptors by fluid movement within the tubules are two theories which involved in dentin sensitivity. The latter is known as the hydrodynamic theory of dentin sensitivity which results in an inward or outward movement of tubular fluid. In addition to a

variety of stimuli, it is affected by tubular radii within dentinal tubules. Fluid movement is proportional to the radius according to equation of Poiseuille's law which refers to functional value rather than the anatomic value, thus changes in tubular radius lead to changes in the rate of fluid movement. Moreover decrease in the functional radius of the dentinal tubules leads to reduce the rate of fluid flow and dentin sensitivity (Greenhill and Pashley, 1981).

$$J_v = \frac{P_1 4}{8\eta_1}$$

Where: $J_v$ = volume flow rate, in $cm^3\ sec^{-1}$;
$P$ = hydrostatic pressure, in dyne $cm^{-2}$;
$\eta$ = viscosity, in dyne sec $cm^{-2}$;
1 = length of tube, in cm;
r = radius of tube, in cm.

**Figure 1** Equation of Poiseuille's law (Greenhill and Pashley, 1981)

The number of dentinal tubules increases with the dentin depth which varies from 15,000 at the DEJ to 65,000 at the pulp (Garberoglio and Brannstrom, 1976; Walton *et al.*, 1976; Mjor and Nordahl, 1996). Koutsi *et al*. (1994) found the greater density and diameters of the tubules in permanent dentition and implied that primary dentition had lower permeability, thus the wetness on dentin surface of the primary teeth is lesser (Nor *et al.*, 1997). On the contrary, Sumikawa *et al*. (1999) found that the tubule density and tubule diameter were greater in primary teeth especially in

anterior primary teeth and the tubule densities were decreased toward the root (Marshall, Jr. *et al.*, 1997). Besides Sumikawa *et al.* (1999) found that the presence of microcanals was prevalent in primary teeth. These microcanals had the roles in inducing higher wetness, being source of tooth sensitivity, increasing the rate of carious attack, and decreasing the area of solid dentin, therefore, decreasing the bond strength of the primary teeth. Permeability of dentin is classified into two types: transdentinal movement which hydrodynamic stimuli influence in fluid movement and intradentinal movement of exogenous substances into intertubular dentin which occurs during resin bonding (Kinney *et al.*, 1995). The rate of diffusion of exogenous material across the dentin to the pulp depends on dentin thickness and the hydraulic conductance of dentin (Pashley, 1991b; Kinney *et al.*, 1995). The thicker the dentin, the lesser substances can diffuse. The permeability of dentin is varied, the permeability in the coronal dentin is higher than in the root since the tubule densities in the root is lower (Pashley, 1991; Kinney *et al.*, 1995). The permeability of sclerotic dentin which results from physiologic or pathologic processes is very low because of mineral deposition in the tubules.

The factors influence on the permeability both in coronal and root dentin are similar. The factors such as dentin thickness, the number of tubule density, and the diameter influence on it (Fogel *et al.*, 1988; Pashley *et al.*, 1993). The tubule density are increased toward the pulp like in coronal dentin, thus the highest tubule density is found in the predentin surface at the junction to the pulp chamber while the lowest tubule density is found at the DEJ (Marshall, Jr. *et al.*, 1997). The number of tubules are constant from the outer root dentin to the root canal, but the tubules are crowded at the canal (Fogel *et al.*, 1988). Although several studies reported variable tubule

density in various region of the teeth, few studies reported in specific and known depths in relation to the DEJ (Sumikawa *et al.*, 1999). However the permeability of root dentin and coronal dentin is not uniform (Galvan *et al.*, 1994). From the study of Pashley (1988), he compared the coronal and radicular hydraulic conductance of dentin and found that at the same dentin thickness, the coronal dentin had higher hydraulic conductance values than the radicular dentin (Pashley, 1988).

Others factors that relate to dentin permeability are smear layers and smear plugs which are composed of debris resulting from restorative or endodontic procedures. They play a role in lowering the permeability whether the presence of the debris is in the tubule orifices or on the dentin surface (Pashley *et al.*, 1978; Pashley, 1984). These influence on restorative procedure using adhesive systems by acid etching (Pashley *et al.*, 1978). The etching procedure increases dentin permeability and dentin wetness by removing smear layer and some of inorganic material dentin at the surface. This will enhance the outward flow of dentinal fluid result in more fluid appear on the surface of dentin. Thus, the effects of the acids used for dentin conditioning may be influenced by the presence of liquids inside the dentinal tubules. However, if sufficient etching time is permitted, the smear layer must be removed from the intertubular dentin, opening the dentinal tubules for the formation of hybrid layer, which is ultimately responsible for strong and stable adhesion of composite resin to dentin (Nakabayashi, 1985; Gwinnett and Kanca, III, 1992; Van *et al.*, 1993).

This study we tried to simulate in vivo conditions, diamond bur was used with copious water irrigation to prepare the dentin surface for bonding procedure (Nor *et al.*, 1997). Whenever tooth structure is cut, reduced dentin thickness results in an

increased diameter and a number of the dentinal tubules close to the pulp tissue and consequently higher dentin permeability. This mechanism is enhanced by the smear layer removal and structural alteration of the dentin tissue caused by the acid application (Hebling *et al.*, 1999).

**2.4 Pulpal pressure**

Dental pulp is surrounded by dentin walls which are rigid due to its low compliance thus, limits the chances to expansion. Tissue pressure or interstitial fluid pressure is the hydrostatic pressure in the interstitial fluid surrounding the pulpal cells which is lower than the blood pressure inside the vessels (Heyeraas and Berggreen, 1999). An increase in both interstitial fluid volume and blood volume which result from inflammation can also increase the tissue pressure. When tissue pressure is increased, increased of lymph flow and absorption of fluid into capillaries nearby noninflamed tissue are initiated, the fluid is then move out from the affected area and the dentin surface. These promote outward flow of fluid through exposed dentinal tubules that may affect to tooth-colored restorative material e.g. dislodgement of restoration and help to protect the pulp from toxic substances that will diffuse into the dentinal tubules. Moreover stimulation of the inferior alveolar nerve can also cause an increase in the rate of outward flow of fluid from the dentin (Heyeraas and Berggreen, 1999). Thus, it can be assumed that pulpal pressure influences the permeability of dentin. Sauro *et al.* (2007) studied on dentin permeability in dental adhesive (HEMA- and free HEMA-based adhesive) and adhesive bond strength due to pressure application. They found that HEMA-based adhesives, which consist of

hydrophilic polymers, were more susceptible to permeability which could see from the fluid flow rate and the number of fluid droplets that appeared on the surface of the resin-bonded dentin. Therefore, the reduction of dental adhesive bond strength occurs. Thus they concluded that bond strength of adhesives would be reduced due to the application of pulpal pressure.

Several techniques have been developed to measure pulpal interstitial pressure. The pressure that has been determined during homeostasis and inflammation can lead to an understanding of vascular responses to pulpal injury. These methods include photoelectric methods (Upthegrove *et al.*, 1968), pressure transducer systems (Brown and Yankowitz, 1964), tonometric measurements (Christiansen *et al.*, 1977), and micropuncture technique (Tønder and Kvinnsland, 1983; Heyeraas, 1989; Heyeraas and Berggreen, 1999)

Most previously mentioned methods recorded the tissue pressure approximately 16-60 mmHg, however they were invasive and traumatized the pulp (Brown and Beveridge, 1966; Upthegrove *et al.*, 1968; Van Hassel and Brown, 1969; Stenvik *et al.*, 1972). The micropuncture technique is much less invasive as the tip diameters of pipettes are only 2-4 μm (Tønder and Naess, 1978). The studies that used this technique could record tissue pressure about 5-6 mmHg under the controlled conditions (Tønder and Kvinnsland, 1983; Heyeraas, 1989).

The pressure in arterioles and venules of pulpal tissue is also higher than other tissues. Tønder and Naess (1978) used micropuncture technique to record the intravascular pressure and reported that arteriole, capillaries pressure, venule pressure and pulpal pressure were 43, 35, 19 and 6 mmHg, respectively.

As mentioned above, studies have demonstrated that in response to the inflammation, pulpal interstitial pressure was increased (Stenvik *et al.*, 1972; Heyeraas and Berggreen, 1999). Tønder and Kvinnsland (1983) used micropuncture technique to measure interstitial pressure of the cat teeth not only the intravascular pressure mentioned above but also the tissue pressure. They found that the pulpal interstitial pressure in cat dental pulp was 16.3 mmHg at the site of pulpal inflammation, 7 mmHg at a site 1-3 mm. next to inflamed area, and 5.5 mmHg in the control teeth. Moreover they demonstrated that the pulpal interstitial pressure response to pulpal inflammation is restricted to the site of injury and is not distributed throughout the pulp (Tønder and Kvinnsland, 1983).

**2.5 Effect of dentin structure and pulpal pressure to adhesive materials**

As we known, dentin is complex hydrated structure which different forms of dentin can be modified by physiological, aging and disease process. Variations in structural components result in variations in the morphology, the distribution of the structural elements and important properties, available surface area for bonding, hardness or shear or bond strength. In general, bond strength is higher in superficial than in deep dentin because of differences in available solid dentin and moisture content (Marshall, Jr. *et al.*, 1997).

Bonding agents can form chemical bonds with the apatite or collagen components of the dentin structure. The association between adhesive and substrate is important for adhesion. Therefore, the quality of dentin bonding is determined by the wetting ability and the capable of penetration of adhesives into dentin. However,

formation of a hybrid layer by impregnation of monomers into partially demineralized dentin, and subsequent polymerization seems to be the most important factor detemining the bonding strength (Marshall, Jr. *et al.*, 1997).

One type of dentin is transparent dentin which results from altered mineralization and structure resulting from caries and irritation. Its characteristic is hypermineralization which leads to occlusion of dentinal tubules and lead to increasing in hardness compared to normal dentin. From the studies of Pashley (1985) and Tagami *et al*. (1992) found that transparent dentin plays a role in the decrease of dentin permeability (Pashley, 1985; Tagami *et al.*, 1992). This acts as a barrier to the penetration of substances, thus it may also impede the penetration of adhesive into the dentin. Moreover, a study by Marshall, Jr. *et al.* (1997) stated that direction of dentinal tubules seemed to have a role in demineralization of sclerotic lesions near the cervical margin because the rate of acid etching of dentin seems to be higher when the acid diffuse down dentinal tubules. However, from several studies whether the studies of Harnirattisai *et al.* (1993) or Van Meerbeek *et al.* (1994) assumed that the adhesion of resins to sclerotic dentin would be weaken because of the low response to acid demineralization or repeated cycles of demineralization and remineralization (Yoshiyama *et al.*, 1996).

In addition, the pulp which contains pressure and surrounds with rigid wall of dentin can also affect to the adhesion of the filling materials, especially tooth-colored restorative materials e.g. composite resin. As seen from a study of Konishi *et al.* (2002) which determine the ultimate shear bond strength at different distances from the pulp showed that these two factors, dentin structures and pulpal pressure, affect to

the bond strength of dental materials. The closer the pulp, the lower ultimate shear bond strength (Konishi *et al.*, 2002). Sauro *et al.* (2007) investigated the effect of intrapulpal pressure on the microtensile bond strength of adhesives, 2-step and 1-step self-etch adhesive systems and concluded that resin-dentin bonds are susceptible to fluid permeation induced by pulpal pressure. There were a correlation between the relative permeability of adhesives and the number of fluid droplets on the adhesive surfaces, therefore, bond strength was reduced due to the application of pulpal pressure (Sauro *et al.*, 2007).

Since these above studies were done in permanent teeth and the structure of dentin in primary and permanent dentition are different, it is possible that the performance of dental adhesives may also be different between them (Kaaden *et al.*, 2003).

**2.6 Dental adhesives**

Dental adhesives are one choice of restorative materials that provide micromechanical retention by infiltration of adhesive resin to the exposed dentinal tubules. In addition, they reduce marginal leakage and recurrent caries, preserve the tooth structure from cavity preparation when used to bond composite to dentin (Swift, Jr., 2002).

Because dentin is more complex structure than enamel, the most highly mineralized tissue, thus bonding to dentin is much more difficult. The strength of the

bonds at the adhesive dentin interface is also affected (Swift, Jr., 2002; Itou *et al.*, 2003).

Dental adhesive systems are divided into 2 systems, etch-and-rinse adhesives (2-step or 3-step) and self-etch adhesives. The differences between them include the adhesive formula which is hydrophilic and ionic resin monomers are incorporated and the facilitating bonding to a wet dentin substrate as well as the elimination of final bonding step. In addition, etch-and-rinse types differ from self-etch adhesive which the former remove the smear layer before application of the resin, while the latter, the smear layer is incorporated (Swift, Jr., 2002).

Etch-and-rinse adhesives are divided into 3-step and 2-step etch-and-rinse adhesives. They consist of 30-40% phosphoric acid, which remove the smear layer and demineralize the dentin to expose the collagen fibrils, and hydrophilic primer to infiltrate the collagen matrix and tubules (Swift, Jr., 2002). The 2-step etch-and-rinse contains a mixture of hydrophilic and hydrophobic resin monomers which are dissolved in volatile organic solvents that causes the simplified adhesives to behave more like a hydrophilic primer than a hydrophobic sealing film. While, the 3-step etch-and-rinse adhesive produces the bonds that are quite strong, durable and impermeable, the system still has problems from postoperative sensitivity due to incomplete sealing of dentinal tubules and technique sensitivity (Swift, Jr., 2002).

Wet-bonding technique has advantage for this system by preventing the collapse of collagen fibrils after demineralized dentin with acid etching, promoting infiltration of adhesive monomers into the acid-etched dentin and giving higher bond strength than dry bonding. Because the presence of water during bonding to dentin

helps to expand the dentin matrix, thus HEMA is one of agents added into primer solution to prevent excessive drying of the dentin surface, promote re-expansion of the collagen network, form hydrogen bonds with water, enhance resin infiltration including improve the final bond strength of the adhesive by strengthening the hybrid layer (Swift, Jr., 2002; Pashley *et al.*, 2007). A bonding agent (Single Bond, 3M ESPE) with etch and rinse approach was used in this study. This contains HEMA so bonding could be affected by the mechanisms explained above. In addition to hydrophilic monomer (HEMA), polyalkenoic acid copolymers are incorporated into the structure to reduce its moisture sensitivity and better stability over time. Reversible breaking and reformation of calcium-polyalkenoic acid complexes in the presence of water suggested developing a stress-relaxation capacity without rupture of adhesion at any time (Kato and Nakabayashi, 1998). Furthermore, ethanol or acetone which is water-chasing agents is also added to aid in the displacement of water from the dentinal surface and the moist collagen network including facilitate the penetration of the resin monomers into the collagen network (Swift, Jr., 2002). Acetone presents a higher capacity to displace moisture from the dentin surface by promoting intense water evaporation, while alcohol and water are less effective (Moll and Haller, 2000; Lopes *et al.*, 2006). This evidence can explain the negative influence of intrapulpal pressure simulation on the bond strength of Single Bond which is alcohol-based adhesive.

Pashley *et al.* (2007) found that the highest tensile bond strength of resins to dentin and the least nanoleakage were seen in 35% HEMA/65% ethanol because the mixture of them produced more resin infiltration.

However, if the excess wetness (overwet phenomenon) was occurred, it contributed to phase changes in ethanol which was very sensitive to dentin's dryness (Bolanos-Carmona *et al.*, 2006). Acetone based resin and forming globules of water leaded to a decrease of bond strength (Agostini *et al.*, 2001). Moreover, acid etching would larger the tubule lumens, which may affect the adhesive bond strength, particularly on primary tooth due to the reduction in the proportion of available solid dentin for bonding (Sumikawa *et al.*, 1999).

Etching time is another factor affecting to bond strength of dentin. Bolanos-Carnoma *et al*. (2006) determined the influence of different of etching times (5, 15 or 30 s) on the microtensile bond strength of primary dentin and found significant differences in lower microtensile strength in specimens etched for 5 s than for 15 or 30 s. No significant difference were found between 15 and 30 s of etching time. Malferrari *et al*. (1995) found that no differences on tensile bond strength when 15 to 120 s etching time were used and the relationship between etching time and tensile bond strength was not found. However, excessive etching times leading to deep infiltration of the adhesive into dentin may lower the dentin modulus. Therefore, 15 s of etching time was suggested to be the proper time (Bolanos-Carmona *et al.*, 2006).

In addition, other causes that increase the greater tubule density in primary teeth included a decrease of solid dentin which may cause difference in bond strength, and the etched peritubular dentin rapidly during bonding treatments, and the left of etched intertubular dentin matrix with enlarged tubule lumens may affect to the bonding efficacy. Thus, understanding the dentin substrate may lead to improved bonding technique (Sumikawa *et al.*, 1999).

Self-etching adhesives, which are developed to shorten the bonding protocols and to reduce the sensitivity of the bonding technique, use chemically modified acidic monomers that are able to demineralize and penetrate dental hard tissues (Kaaden *et al.*, 2003). Thus, removal of smear layer is not required in this system (Swift, Jr., 2002). Their active components are esters from bivalent alcohols with methacrylic acid and phosphoric acid or derivates. The phosphate residue causes the conditioning of enamel and dentin, similar to the effect of phosphoric acid, while the methacrylate component of the molecule is available for copolymerization with the adhesive or the restorative material (Kaaden *et al.*, 2003). In addition, some of functional monomers can interact with hydroxyapatite, thus improve the durability of the resin-dentin bond. Another important component is 30-40% water which plays a role in generating hydrogen ions for effective demineralization of the smear layer and the underlying dentin (Swift, Jr., 2002).

Self-etching adhesives are classified into mild (pH>2), moderate (pH 1-2) and aggressive (pH<1) self-etching adhesives depending on the ability to penetrate dentin smear layers and the depth of demineralization (Swift, Jr., 2002). Mild and moderate type provide the depth of hybrid layers approximately 0.5-1 μm (Swift, Jr., 2002; Pashley *et al.*, 2007), which differ from etch-and-rinse adhesives that produce about 5 μm of hybrid layers thickness (Takahashi *et al.*, 2002). Aggressive type has bonding mechanism to completely dissolve the smear layer and form the hybrid layer thickness like etch-and-rinse adhesives (Takahashi *et al.*, 2002).

Since there are differences between primary and permanent dentin, the study of Nör *et al.* (1997) and Olmez *et al.* (1998) stated that acid conditioning of primary

dentin, which is more reactive to acidic conditioners, causes the formation of a 25-30% thicker hybrid layer compared with permanent teeth. Therefore, they suggested a different protocol with shorter acid conditioning times when bonding to primary teeth. However, the instructions for use when treating primary teeth are not firmly established and currently manufacturers do not recommend a different protocol for their products when used for bonding to primary teeth (Kaaden *et al.*, 2003).

**2.7 Bond strength tests**

Methods in testing for adhesive materials are conventional method, shear bond strength and tensile strength tests, and microtensile bond strength test (Takahashi *et al.*, 2002). Microtensile strength test which we used in this study has advantages above conventional method in using less bonded area for testing and having more stress distribution during testing (Takahashi *et al.*, 2002) which affects to failure mode that most specimen present cohesive fracture (Cardoso *et al.*, 1998). Although it is more labor-intensive than conventional testing, but holds great potential for providing insight into the strength of adhesion of restorative materials to clinically relevant sites and substrates (Pashley *et al.*, 1999). The use of small adhesive interface in microtensile strength test which has been developed to permit measuring cross-sectional bonded areas as small as 0.5 $mm^2$ (Sano *et al.*, 1994) leads to have less defect than large bonded interface used in conventional method, thus the value of bond strength is increased and the variation is reduced (Cardoso *et al.*, 1998). Moreover, the microtensile strength test permits the testing of irregular surfaces such as class I, II and V restorations and can be used to compare the regional bond

strength of occlusal and gingival floors of wedge-shaped cervical defects (Yoshiyama *et al.*, 1996).

Conventional method either the tensile or the shear test method can be used for quality testing of dentin adhesives (Oilo and Austrheim, 1993). Shear bond strength test can be proper method when forces approximately 18-20 MPa are applied. But if the force is excess, cohesive failure in dentin which rarely found and can be minimized in microtensile testing is increased (Schreiner *et al.*, 1998; Scholtanus *et al.*, 2010). The microtensile strength test was used more often than shear bond strength tests due to its results correlating to the clinical feature and less dentin failure (Schreiner *et al.*, 1998). Moreover, adhesive failure can be found in microtensile strength test more than shear bond strength test, thus it may be used in bond strength evaluation of materials better than the other (Schreiner *et al.*, 1998). Furthermore, tensile force which results in clinical failure is used in microtensile test more than shear force, therefore, microtensile test should be used in clinical evaluation of restorative materials (Schreiner *et al.*, 1998). Another observation from the study of Schreiner *et al.* (1998) found that the results from microtensile strength test of dentin adhesive systems could be found differences between them, while shear bond strength test didn't found.

The characteristics of microtensile strength test which was developed by Sano *et al.* (1994) are geometry at the bonding interface, more uniform stress distribution across the interface and more accurate assessment of bond strength of adhesive materials. This test is less extensively than shear or tensile bond strength tests (Betamar *et al.*, 2007). It is recommended that bonded area for testing should be

approximately 1.5-1.8 $mm^2$ (Schreiner *et al.*, 1998; Betamar *et al.*, 2007). The shapes of specimens used in the test included stick shape which is the first shape used, dumbbell shape which is the most used (Scholtanus *et al.*, 2010), and hourglass shape. However, the potential effects on the microtensile bond strength of the differences between the three specimen shapes are unknown (Betamar *et al.*, 2007). In addition, from the study of Phrukkanon S. *et al*. (1998) in comparing cylindrical cross-sectional specimens with the rectangular specimen shapes for a microtensile test found that the bond strength had no difference within the same bonding systems between rectangular and cylindrical specimens. Moreover, cross-sectional shape had little effect on bond strength of 1.5 $mm^2$ surface area when compared with the smaller areas (1.1 $mm^2$) where the results from both are almost identical. Furthermore, cylindrical specimens can distribute the stresses along the bonding surface better than rectangular specimen. This study recommended that the size of 1.5 $mm^2$ and cylindrical-shaped specimen are proper for microtensile testing.

There are several reports about bond strength testing of adhesive materials in primary and permanent dentin. Although these results are variable (Agostini *et al.*, 2001; Bolanos-Carmona *et al.*, 2006), most of them studied in permanent dentin. However, the studies on microtensile bond strength on primary dentin particularly in relating with dentin permeability are scarce and difficult to compare because of differences of methods or materials (Bolanos-Carmona *et al.*, 2006).

Besides bond strength testing is a useful tool for comparison of adhesive properties in the different substrates. For example, the study of Scholtanus *et al*. (2010) found the interaction between bonding system and the quality of the dentin.

They found that bond strength values were different between normal and caries-affected dentin which had lower bond strength to adhesives. However, results of microtensile bond strength tests cannot implied to see the effectiveness of adhesive systems in clinical since the intraoral conditions which has a variety of continuous and intermittent mechanical, thermal stress and chemical challenges including aging which might have a deteriorating effect on bonding quality.

In addition, The modulus of elasticity of the resin composite has been shown to influence the results of bond strength measurements (Moll *et al.*, 2005). Thus, the same composite material and shade were used in order to avoid composite-related influences on bond strength.

**2.8 Thermocycling**

The difference between the linear coefficients of thermal expansion of the tooth and the restorative material is a factor of the integrity of the tooth/composite restoration interface. Thus, thermocycling is the *in vitro* process which simulates the introduction of hot and cold extremes in the oral cavity and shows the relationship of the linear coefficient of thermal expansion between tooth and restorative material. Wendt SL *et al.* (1992) investigated the effect of thermocycling on dye penetration during in vitro microleakage analysis of composites. They found that no significant increase of microleakage in restorations when thermocycling was used to simulate temperature extremes, as opposed to restorations which were not thermocycled. This corresponds to Li H *et al.* (2002) who found that thermocycling does not have an

effect on nanoleakage of dentin bonding systems. Thus, thermocycling was not included in the process of this study.

Therefore intraoral conditions whether previously mentioned or pulpal pressure which was investigated in this study, should be considered in the studies of adhesive bond strength which will provide more reliable information in effectiveness of adhesive systems.