CHAPTER 2

Literature Review

2.1 Pulp biology

Dental pulp is a special tissue, composed of many tissue elements, including axons, vascular tissue, connective tissue fibers, ground substance, interstitial fluid, odontoblasts, fibroblasts, immunocompetent cells, and other cellular components. The odontoblast is the most specialized cell, which directly contacts the dentinal tubules and forms the dentine-pulp complex.

The relationship of dentine and pulp begins when the tooth is formed (9). Primary dentine is formed by the odontoblasts during the development of the tooth. Primary dentine is formed by the odontoblasts during the development of the tooth. After that, the odontoblasts continue to deposit secondary dentine throughout the life of the tooth. When the tooth is subjected to occlusal wear or disease, dentine is laid down on the pulpal aspect and is called tertiary dentine. There are two types of tertiary dentine: reactionary dentine and reparative dentine. The dentine-pulp complex is very important when dentine is exposed. Many factors, such as pulpal innervation, pulpal blood flow, intravascular and tissue pressure, dentine permeability and dentinal fluid flow via dentinal tubules are involved in this complex. All of these factors are causes of dentine sensitivity. Dental pulp is surrounded by the rigid enamel and dentine; as a result, it has a very low compliance. When there is external stimulation, which causes vasodilatation, the pulp has no ability to expand and the volume of the pulp chamber cannot be increased. This characteristic of the dental pulp is different from most other tissues.

2.2 Pulpal innervation

The pulps of the teeth are innervated by branches of the trigeminal nerve, which include both sensory afferent and postganglionic sympathetic axons (10,11). The trigeminal neurons have their cell bodies in the trigeminal ganglion and are primarily

sensory in function. The nervous system is composed of both afferent neurons, which conduct sensory impulses, and autonomic or efferent neurons, which provide neurogenic modulation of the microcirculation, inflammatory reactions, and regulate dentinogenesis (9). There are two types of sensory nerve fibers in dental pulp: myelinated (A fibers) and unmyelinated (C fibers). The A fibers include A-beta and A-delta fibers. A-delta fibers comprise approximately 90% of the A fibers in the dental pulp. The A-beta fibers may be slightly more sensitive to stimulation than the A-delta fibers. These fibers are grouped together in the dental pulp and they innervate the dentinal tubules, and are stimulated by dentinal fluid movement. The A fibers are more sensitive to cold stimuli and heat stimuli than C fibers. Most stimuli excite nerves indirectly, via a hydrodynamic mechanism. C fibers are not activated by these stimuli unless they produce injury to the pulp.

When external stimuli are applied to human dentin, it causes pain, and the fluid flow through dentine increases (12). Some stimuli increase fluid flow outwards from the pulp, such as cooling, drying, evaporation, hypertonic solutions and decreased hydrostatic pressure, while others produce inward flow, such as heating, mechanical stimulation and increased hydrostatic pressure. Matthews and Vongsavan found that intradental nerves are excited more readily by stimuli that produce fluid flow outwards from the pulp, which generates greater action potentials when the flow is directed outwards rather than inwards (13).

The sympathetic innervation of teeth derives from the superior cervical ganglion. Postganglionic sympathetic nerves travel with the internal carotid nerve, join the trigeminal nerve at the ganglion, and supply teeth and supporting structures via the maxillary and mandibular divisions of the trigeminal nerve (14). There is no conclusive evidence for a parasympathetic innervation of pulp.

2.3 Vascular supply

The vitality of the pulp depends on vascular supply (15). The pulp is supported by a microcirculatory system. It is composed of arterioles and venules, which pass through the apical foramen and accessory foramina together with nerve bundles. Both arterioles and venules pass axially in the root pulp towards the coronal pulp. Venules align in the

peripheral part of the pulp, whereas arterioles are more central. The small vessels leave the central pulpal arterioles, connect with venules and pass towards the peripheral pulp and form an extensive terminal capillary network. The capillary network extends up towards the odontoblast layer to the surface of the pulp (12). Almost 90% of the capillary network is found in the subodontoblastic region. This network does not pass into the dentinal tubules. It provides the odontoblasts with a rich source of metabolites.

The structure of blood vessels in the pulp is similar to that in other tissues, but the vessel wall is thinner than that in other tissues (16). The thin-walled capillaries facilitate the exchange of nutrients and waste products between the interstitial tissue fluid and the blood plasma. This is important when the dental pulp is injured by dental procedures, trauma or dental caries. The lymphatic system promotes the transportation of fluid out of the pulp and then the inflammation reduces.

2.4 Pulpal blood flow

Dental pulp has a high resting blood flow due to its highly vascular supply (17). Pulpal blood flow is controlled by the nervous system. The mechanisms of this control are similar to those in other tissues. Pulpal blood flow is also influenced by the vascular tone in neighboring tissues. Stimulation of the sympathetic nerve supply to the pulp results in the reduction of pulpal blood flow, while stimulation of afferent nerves can increase pulpal blood flow (13).

Stimulation the dental pulp with vasodilator agents increases pulpal blood flow and stimulation with vasoconstrictor agents decreases pulpal blood flow (18-20). Injection of local anesthetics, which contain a vasoconstrictor, adrenaline or epinephrine, at the apex of the root significantly decreases pulpal blood flow (18).

Although pulpal blood flow is very difficult to measure accurately, changes in pulpal blood flow can be measured through dentine using a laser Doppler flow-meter (7,21). The resting blood flow in the pulp is relatively high, 0.15 to 0.17 ml/min/g, which is in the same range as blood flow in cerebral white matter and four times as high as in resting skeletal muscles (17). The high blood flow may reflect the high metabolic rate of the pulp cells or may be a consequence of the low compliance of the dental pulp.

Capillary blood flow in the coronal portion of the pulp is nearly twice than in the root portion (9). Moreover, blood flow in the region of the pulp horns is greater than in all other areas of the pulp and pulpal blood flow in the peripheral layer of the pulp is about four times greater than in the central pulp.

2.5 Intravascular and tissue pressures

The microvascular pressure is the blood pressure within the different vessel segments, arterioles, capillaries, and venules. Changes in arteriolar and venular blood pressure causes changes in pulpal blood flow.

Blood is brought to the tissues through the vessels, and substances are transported between blood and interstitial fluid by diffusion (22) (Fig. 2.1). The main function of the interstitial fluid is to transport medium for nutrients and waste products between cells and capillary blood. The difference in protein concentrations between plasma and interstitial fluid induces a colloidal osmotic pressure, thus favoring fluid movement into the capillary. The hydrostatic pressure in the interstitial fluid surrounding the cells is the interstitial fluid pressure (P_i), or so-called tissue pressure.

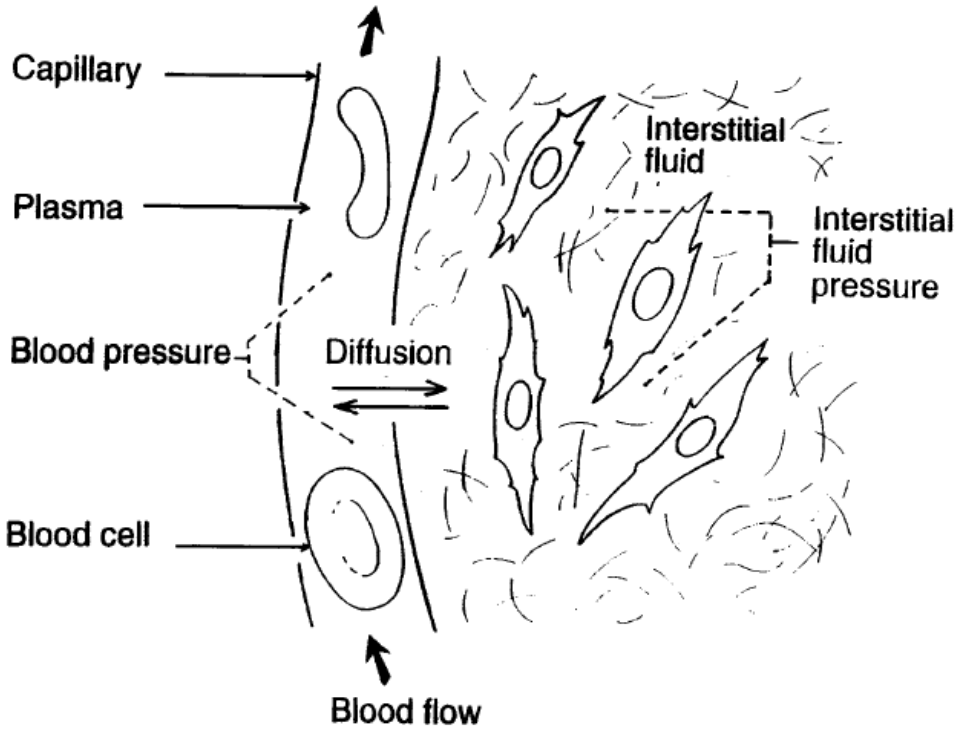

Figure 2.1 Illustration of capillary, cells, and interstitial fluid. The interstitial fluid pressure is the hydrostatic pressure in the interstitial fluid surrounding the cells (22).

The pulpal tissue pressure concerns the hydrostatic pressure exerted by the interstitial fluid in the exstravascular pulpal tissue (22). Tissue pressure may affect blood flow due to the low compliance environment of the pulp and vice versa. Any increase in fluid volume will increase P_i. Extracellular fluid volume can increase in two circumstances: 1) increased blood volume (due to venous stasis or increased blood flow), and 2) increased interstitial fluid volume (IFV). IFV can be increased by an increase in capillary blood pressure or protein concentration in the interstitial fluid which also increases capillary filtration.

Tønder and Kvinnsland (23) measured tissue and blood pressure in the dental pulp by using the micropuncture technique. The advantage of this technique is that it can measure blood pressure in the small vessel segments and can measure tissue pressure in the extravascular tissue. The micropuncture technique produces less trauma during measurement than do the other methods because of the smaller exposure and minute diameter of the measuring pipette. Moreover, it does not add or remove fluid from the

interstitium. This technique may not be appropriate to measure blood flow in the dental pulp, which has low compliance because the pulp must be exposed and tissue is damaged; therefore, recorded pressure may not represent normal physiological values. The average interstitial fluid pressure in healthy teeth of humans varies between 15-30 mmHg(24). This variety of pressure may be due to the low compliance of the pulp. The minute change in pulpal volume, caused by the measuring equipment and/or pulpal exposure, is reflected in the tissue pressure.

Tønder and Naess found that the average pressure in the arterioles is 43mmHg, whereas the average pressure in the venules is 19.8 mmHg (25). The tissue fluid pressure in the pulp is 11-30 mmHg, which is much higher than in most other tissues (23,26).

2.6 Tissue pressure in the inflamed pulp

Acute inflammation of the pulp induces vasodilatation of the pulpal arterioles and an increase in interstitial pulpal fluid and pulpal blood flow followed by increased vascular permeability (23, 26). This may also increase blood volume and the IFV in the inflamed area. Since dental pulp is a low-compliance tissue, an increase in volume may increase the tissue pressure (Fig. 2.2). The tissue fluid pressure in the inflamed area is increased by 8-10 mmHg. Tønder and Kvinnsland (27)suggested that an increase in tissue fluid pressure in an inflamed area of pulp would lead to an increase in tissue fluid uptake into the plasma in the capillaries in an adjacent area of normal pulp which would relieve the pressure on the venules. Because the wall of the pulpal vessel is thin, if the tissue pressure outside the vessel rises to the same level as the blood pressure inside the vessel, the vessel may be pinched off or strangled, thus causing stagnation of the blood circulation, with resultant ischemia and necrosis. The increased tissue pressure also compresses the thin-walled veins and thus lowers blood flow and increases venous blood pressure. A rise in venous pressure automatically increases the capillary blood pressure and thereby capillary filtration, which develops a “vicious circle”. The the self-strangulation theory of pulpitis was found on this vicious circle (Fig. 2.2).

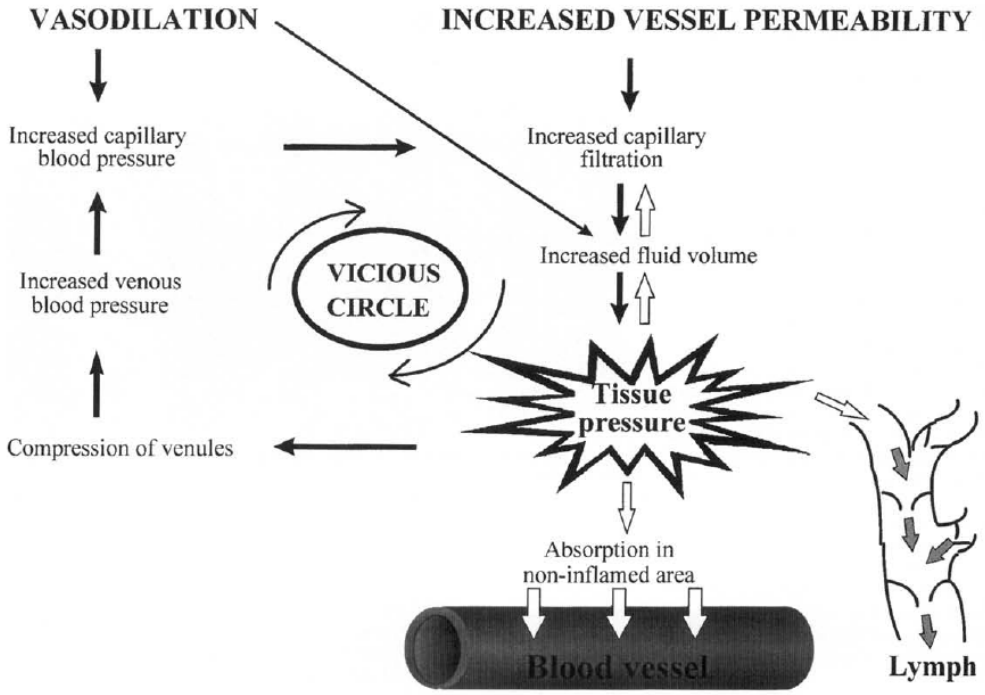

Figure 2.2 Schematic build-up of a vicious circle during pulpal inflammation. The edema-preventing mechanisms that serve to keep the pulpal fluid volume relatively constant, therefore opposing a rise in tissue pressure and breaking the vicious circle, are indicated with open arrows. This vicious circle was the basis for the self-strangulation theory of pulpal inflammation (22).

When dentine is exposed, the dental pulp is inflamed and pulpal blood flow increases. This results in high tissue fluid pressure and promotes the outward flow of fluid through the dentinal tubules. The sensory nerve fibers in the exposed area, which is stimulated by the hydrodynamic movement of fluid into the dentinal tubules might be the cause of vasodilatation and increase in Pi due to the release of neuropeptides via axon reflex (22).

Fluid transport from the inflamed pulp area and out of the tooth results from two possibilities: 1) the lymphatics, and 2) the blood vessels in the adjacent normal tissue (28). An increase in P_i increases lymph flow. The result of increased lymph flow is reflected in the removal of protein, which is important in keeping the colloidal osmotic pressure of the pulp low, and prevents further filtration in the inflamed pulp. As a result,

fluid is transported out of the affected area and then out of the tooth and consequently the tissue pressure is reduced (Fig. 2.2). If the pulp is to survive and heal, lymphatic vessels must remove plasma proteins form the pulpal interstitial fluid.

Another mechanism that can protect the pulp during inflammation is that when P_i increases due to pulpal inflammation, pulpal blood flow increases followed by an increase in the outward flow of fluid through the dentinal tubules (22). This outward flow reduces the rate of inward flow of toxic substances from outside the tooth. Thus, the outward flow of fluid will help to protect the pulp against entry of harmful substances.

2.7 Dentinal fluid

Dentinal fluid is an ultrafiltrate of blood from the pulp capillaries. It comprises plasma in many respects (9). This fluid is approximately 22% of the total volume of dentine. The fluid can flow outward and inward from the odontoblasts in the dental pulp through the dentinal tubules. The outward fluid flow is blocked by enamel on the crown and cementum on the root. It has been shown that the tissue pressure of the pulp is approximately 14 cmH_2O (10.3 mmHg)(29). Consequently, the different pressure between the pulp and the oral cavity causes a slow outward flow of fluid when dentine becomes exposed. The fluid that moves outward to the exposed dentine surface is formed in the tiny droplets (30). Dehydrating the surface of the dentine with compressed air, dry heat, or the application of absorbent paper can accelerate the outward movement of dentinal fluid, causing dentine sensitivity.

Bacterial products from dental caries or bacterial biofilms under restorations may pass into the dental pulp via the dentinal fluid and cause pulpal inflammation. Furthermore, bacteria from necrotic pulp may pass into the periradicular tissue, and then, cause apical periodontitis. In the case of periodontal disease, with bone resorption, dentinal fluid may carry bacterial toxins or noxious agents from outside the exposed root into the pulp, and cause pulpal injury. This is why pulpal necrosis can be found in a case which has no evidence of any dental caries or restorations (1,3).

2.8 Dentine permeability

Dentinal tubules are the main paths by which materials can pass from outside into the dental pulp. Because the amount and rate of fluid flow through dentine is proportional to the diameter and the number of dentinal tubules, dentine permeability increases as the number and the diameter of tubules increase (31). The total tubular surface close to the dentino-enamel junction (DEJ) is approximately 1% of the total surface area of dentine, whereas close to the pulp chamber, the total tubular surface is nearly 45%. The dentinal tubules are slightly tapered, with the wider diameter at the pulpal end. Thus, the dentine in the deep portion of a cavity preparation is much more permeable than that in a shallow cavity. The density of dentinal tubules in the apical portion is much lower than those in the cervical portion (Fig. 2.3). The density of the dentinal tubules in the cervical area is approximately 42,000/mm^2 and 8,000/mm^2 in a radicular area. Fogel and colleagues (32) found that the permeability of radicular dentine is much lower than that of coronal dentine. The low permeability of radicular dentine makes it impermeable to toxic substances, such as toxins from microbial plaque. An inflammatory reaction to dental caries develops in the pulp quite a long period before the pulp becomes infected with microorganisms (33). This means that bacterial byproducts reach the pulp before the bacteria themselves, through the dentinal tubules. Dentinal sclerosis beneath a carious lesion reduces permeability by obstructing the tubules, thus decreasing the delivery of irritants into the pulp.

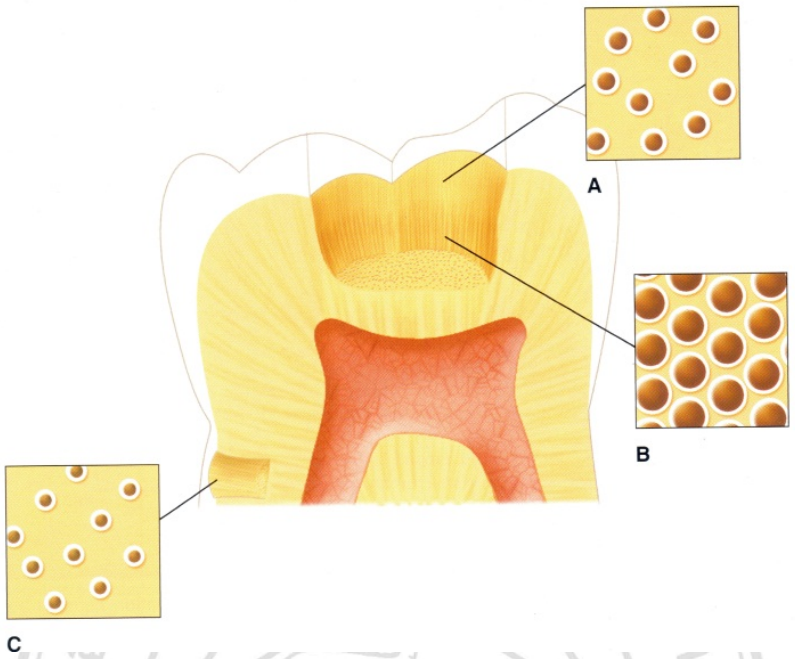

Figure 2.3 Size and density of dentinal tubules. Diagram illustrates the difference in size and density of tubules in the dentinal floor between shallow (A) and a deep (B) cavity preparations, in coronal dentin versus (C) root dentin (9).

Pulpal inflammation after tooth preparation or cavity preparation can be induced by leaving dentine exposed to the oral environment (34). Microorganisms, present on the dentine surfaces, can penetrate into the dental pulp via the dentinal tubules and cause pulpal inflammation (35). Bergenholtz (36) demonstrated that bacterial products can induce pulpal inflammation through freshly cut dentine and found that dentine permeability was the most important factor causing pulpal irritation from dental caries, preparation procedures and dental materials.

The cutting of dentine during tooth preparation produces a smear layer that coats the dentine and clogs the dentinal tubules, preventing bacteria from penetrating the dentine. Removal of the smear layer by acid etching increases dentine permeability by widening the dentinal tubules and can cause pulpal inflammation.

Factors that cause pulpal inflammation after cutting the dentine are not only the reduction of dentine permeability, but also the reduction in the length of the dentinal tubules by tooth preparation. In normal dentine, the dentinal tubules are approximately

3 mm long (37). This long distance dissipates the concentration of bacterial toxins and noxious materials 100- to 1000- fold, so that when they reach the pulp, their concentrations are very low. When dentine is cut, the dentinal tubules are shorter and dentine becomes more permeable, promoting the bacteria, their toxins and materials to penetrate into the pulp easier, faster and with more concentration. This is one reason why the dental pulp becomes injured when a cavity is very deep or a preparation is extensive. However, in normal dentine, there are three sources of resistance to fluid movement in dentinal tubules (30). First, the presence of a smear layer or smear plug accounts for 86% of the total resistance to fluid movement. Second, 7.5% of the total resistance is caused by the presence of odontoblasts and their processes. The remaining 6% of the total resistance is caused by intratubular resistance from the presence of collagen fibers. When dentine is etched, there is only intratubular and pulpal resistance, which comprises less than half of the total resistance. When dentine is cut, the dentinal tubules are shortened and, the intratubular resistance to fluid flow decreases, resulting in an increase in the potential for dentine sensitivity.

Nagaokaand colleagues (38) found that bacteria penetrated into the dentinal tubules of nonvital teeth more rapidly than in vital teeth when the dentine surfaces were exposed to the oral environment for 150 days. This rapid penetration was because the outward movement of dentinal fluid in vital pulp resisted the inward flow of bacteria and toxins into the pulp.

2.9 Fluid flow through dentine

When dentine is exposed, from tooth fracture, dental caries or cavity preparation, dentinal fluid flows outward from the dentinal tubules to the exposed dentine surface. Dehydration of the dentine surface by compressed air, dry heat or the application of absorbent paper can accelerate this outward movement of fluid (9). This is caused by the hydrostatic pressure of the pulpal tissue fluid and the hydrodynamic movement of fluid in the dentinal tubules. The outward flow of fluid through exposed dentine is important because it can reduce the rate of inward diffusion of toxins from the mouth into the pulp. When the dentinal fluid flows outward through the exposed dentine, however, there are some substances in solution from outside diffuse slowly into the pulp. The other important thing is that the outward flow activates pulpal

mechanoreceptors more effectively than the inward flow does (13). It was found that intradental nerves were much more sensitive to outward than inward flow through dentine; as a result, the outward flow of fluid causes greater dentine sensitivity than inward flow does.

2.10 Formation of the smear layer

After cavity preparation or tooth reduction by hand instruments or dental burs, a thin layer of microcrystalline debris is always found on the dentine surfaces. This grinding debris consists of ground components of enamel and intertubular and peritubular matrix, matrix from dentinal tubules, dentinal fluid, and saliva. This layer of debris, which is less than 2 microns thick, clogs the orifices of the dentinal tubules and it is termed the *smear layer*(39).If dentinal tubules are opened as a result of tooth preparation, small particles ofthe smear layer may extend into the dentinal tubules and reduce the fluid flow through dentine; as a result, the dentine permeability decreases. Consequently, it is difficult for bacteria and their toxins or noxious agents to penetrate into the dental pulp.

In many restorative procedures, the smear layer should be removed in order to obtain proper chemical and mechanical bonding between the dentine surface and the adhesive system (9). The opened dentinal tubules allow the resin to infiltrate the tubules. Water spray or scrubbing of prepared surfaces cannot remove the smear layer. Polishing the prepared surface by pumice can remove the smear layer but leaves the smear plug in the opening of the tubules, so that the resin cannot infiltrate into the tubules. To remove the smear layer and open the dentinal tubules, acid-etching agents or ethylenediamine tetra acetic acid (EDTA) can be used. Acid demineralizes the smear layer from the prepared surface and leaves the tubules wide open (Fig. 2.4). This action greatly increases dentine permeability; as a result, the incidence of pulpal inflammation may be increased significantly. When the exposed dentine needs to be treated by acid etching, the pulp should be protected by applying lining materials.

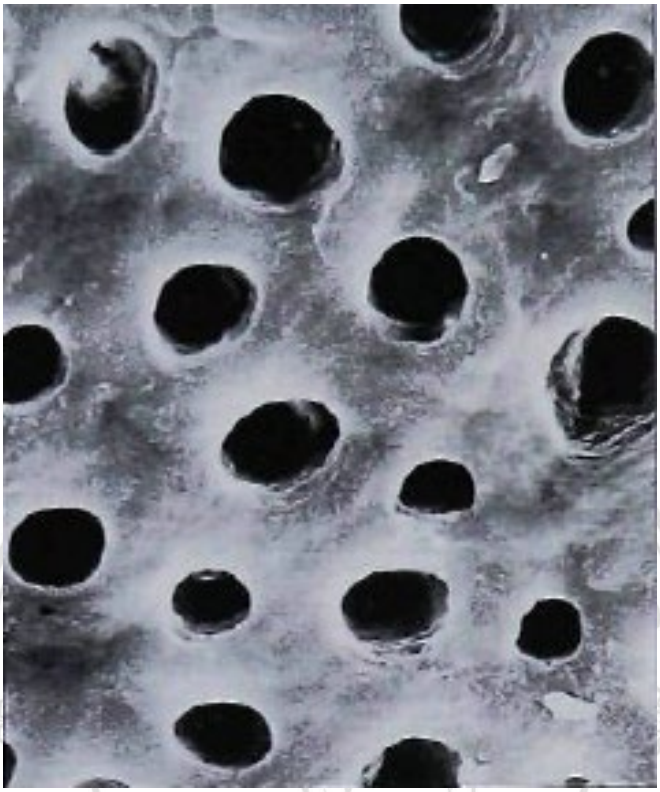

Figure 2.4 Scanning electron micrograph of the ground dentine surface. It has been treated with 35% phosphoric acid for 60 seconds and washed with a water spray. Dentinal tubules have been opened and the peritubular dentine has been demineralized (40).

2.11 Pulpal reactions to dental caries

Dental caries is a progressive infection of dentine. It can cause pulpal infection, pulpal necrosis and tooth loss by bacterial byproducts. Bacterial metabolites, such as acids, are initiators of pulpal reaction, but the dentinal fluid has a capacity to buffer these acids before it can be harmful to the pulp. The tooth has three protective defense mechanisms to protect the pulp against caries: 1) a decrease in dentine permeability, 2) tertiary dentine formation, and 3) inflammatory and immune reactions (41). A decrease in dentine permeability can occur from an increased deposition of intratubular dentine and the direct deposition of mineral crystals into the narrowed dentinal tubules. This is the first defense against caries, and occurs after a short time and is called dentine sclerosis. The formation of tertiary dentine occurs over a longer period than does that of

sclerotic dentine. Tertiary dentine is referred to as *reactionary dentine* and can be observed under noncavitated enamel lesions. Immune reactions can provide humoral and cellular challenges to invading pathogens. When caries progresses, the host immune response increases in intensity as the infection advances. Many mediators and chronic inflammatory cells increase in the early inflammatory response to caries. In advanced phases of caries, the humoral immune response is accompanied by immunopathologic destruction of pulpal tissue.

2.12 Pulpal reactions to local anesthetics

Most local anesthetic agents used in dentistry have vasodilatation properties, which affect the pulpal blood flow after administration. Epinephrine is the most widely used vasoconstrictor in local anesthesia with the concentration in dental procedures of 1:100,000.It is used to prolong the duration of the anesthetic effect, but it has a negative effect on dental pulp by reducing pulpal blood flow, especially if the pulp is already inflamed. Pitt Ford and colleagues (42) compared pulpal blood flow and duration of anesthetic effect after local injection of 2% plain lignocaine and lignocaine with 1:80,000 adrenaline in maxillary central incisors in human subjects. They found that pulpal blood flow significantly decreased after injection with lignocaine with 1:80,000 adrenaline, but did not change with plain lignocaine. The duration of pulpal anesthesia following injection with lignocaine with 1:80,000 adrenaline was four times longer than with plain lignocaine.

Odor and colleagues (20) studied the effect of adrenaline on pulpal circulation using a Laser Doppler flow meter. They found that, there were significant decreases in pulpal blood flow after injection mandibular nerve block with lignocaine with adrenaline, both 1:100,000 and 1:80,000, in human canines and molars. The higher concentration of adrenaline produced a longer duration of anesthetic effect than the lower concentration. The duration of blood flow reduction was shorter with the lower concentration of adrenaline. However, there was no difference between the two vasoconstrictor solutions for the magnitude of the reduction in pulpal blood flow.

Kim and colleagues (18) also found that vasoconstrictors in local anesthetics (2% lidocaine with 1:100,000 epinephrine) reduced pulpal blood flow in animals when

administered by infiltration or mandibular nerve block. Furthermore, they reported that this effect was more severe with periodontal ligament injections (43).

Since the dental pulp is a low-compliance tissue, it cannot change its volume to compensate for the change in blood flow. Reduction in blood flow affects the ability to clear the large molecular-weight toxins or waste products, thus causing irreversible pulpal pathosis. The reduction in pulpal blood flow from local anesthesia, therefore, compromises the ability of the inflamed pulp to recover from inflammation, particularly in severely inflamed pulp, in a tooth subjected to extensive restorative procedures or in a tooth administered a periodontal ligament or intraosseous injection.

2.13 Pulpal reactions to cavity, crown and bridge abutment tooth preparation

Many restorative procedures such as fillings, inlays, onlays, veneers, crowns and bridges and the materials used, such as filling materials and dental cements can cause significant irritation to the pulp. The tooth preparations for fillings, inlays, onlays and crowns are usually performed on teeth with previous extensive caries. Some defects are deep and very close to the dental pulp. Further preparation to remove caries and prepare the cavity for restoration can cause pulpal irritation and pulp necrosis.

In the process of tooth preparation, which is one of noxious stimulation, inflammatory mediators and neuropeptides are released. These mediators change vascular functions; as a result, blood flow in the dental pulp increases. The increase in pulpal blood flow promotes the ability to clear the inflammatory mediators and helps the pulp to recover from inflammation.

Bidar and colleagues (44) studied the effect of full crown preparation on normal and inflamed pulp tissue in cats. They prepared class V cavities in cat canines and filled soft decayed dentine in the experiment group for one month and then prepared a full coverage crown. One month later, the histologic evidence in the pulp showed a necrotic change in 67.8% of all teeth, with no significant difference between intact and inflamed pulps. Pulpal injury still occurred after tooth preparation, even in sound teeth.

Jackson and colleagues (45) demonstrated that only 5.7% of teeth showed pulpal injury after preparation for full coverage crown in humans, whereas Cheung and colleagues (2) showed pulp necrosis in up to 32.5% of teeth prepared for bridge abutments.

Since full crown preparation exposes the dentine and the dentinal tubules to a greater extent than do the other preparations, the possibility of pulpal injury is greater. Dahl (46) demonstrated that a dentine smear is formed when preparing the tooth with a water-cooled diamond at ultra-high speed. Brannstrom and Johnson (47) found bacteria, which may irritate the pulp, on prepared dentine surfaces. They reported that the dentine smear which contains bacteria can be removed by scrubbing with a microbicidal solution. However, the smear plug from the grinding debris which clogs the dentinal tubules decreases the dentine permeability and reduces an inward flow of bacterial toxin into the pulp.

The pressure and heat generated during tooth preparation irritate the dental pulp. Langeland and Langeland (48) found histologic changes in dental pulp after full crown preparation without sufficient water coolant. Displacement of odontoblasts and disorganization in the organelles of the odontoblasts were observed after excessive tooth preparation. Many factors affect pulpal response after full crown preparation: the use of water coolant, the amount of pressure applied, the heat generated, previous caries, the remaining dentine thickness and microleakage of the restoration.

2.13.1 Water coolant, heat, and pressure

Preparation procedures with dental burs in an airotor handpiece can cause pulpal irritation if the preparation is done with insufficient water spray or under pressure because heat is generated under these two conditions. Kim and colleagues (49) investigated the effect of drilling dentine on pulpal blood flow. Their experiments were carried out in dogs using an invasive technique, in which blood flow was estimated by injecting radio-labeled microspheres into the circulation and counting the numbers trapped in the pulpal capillaries. They found that, with a water spray, drilling dentine caused a reduction in PBF of around 13% from the baseline, resting value. Without

water spray, the corresponding value was a 34% fall, and it fell to 89% below baseline one hour after the preparation.

The heat generated during preparation can be reduced by air or water. Many studies (2,3,48,50) suggest cutting the tooth structures with adequate water spray coolant rather than air coolant to avoid desiccation of the dentine. The direction of the water spray is very important. Even if sufficient water spray for cooling is used, in fact, the dentine remains dry due to the deflection of the water from the bur. Langeland and Langeland (51) obtained histological evidence that crown preparation in human teeth produces no initial pulp reaction as long as it is carried out with adequate water spray. They found that if the water spray was insufficient, the dentine showed evidence of burning, and odontoblast cell bodies were displaced into the ends of the cut dentinal tubules. This indicated the disintegration of the odontoblasts. The histologic change in odontoblasts appeared more severe when the preparation was done under pressure, increased temperature and vibration. They concluded that it was very important to provide sufficient cooling to the bur. They postulated that as a result of such cooling, the temperature in the dentine would not increase and the burned lesions in the histologic sections would not have been presented.

Zach ad Cohen (52) showed that if the temperature in the pulp chamber increased 5.5 ^{O}C, the pulp could be damaged, resulting in complete loss of vitality in 15% of teeth. The temperature changes were influenced by water flow, air pressure and applied load. Öztürk (53) demonstrated that the temperature in the pulp significantly increased 7.1 ^{O}C to 19.7 ^{O}C when the teeth were prepared without water cooling, but decreased insignificantly 1.8 ^{O}C to increased 3.1 ^{O}C, when prepared with copious water cooling.

Langeland and Langeland (51) found that when the speed of the cutting instruments increased from 10,000 rpm to 200,000 rpm, the water deflected from the bur. They also found that the deflection occurred more often when using high speeds than low speeds. The direction of the water spray outlet is very important, since the water should reach directly to the dental burs in order to reduce heat effectively during preparation.

The speed of the airotor handpiece affects the reaction of the dental pulp during preparation (51,54). Swerdlow and Stanley (54) showed that when dentine was cut with very low speeds of up to 300 rpm, with sharp burs and low pressure, minor, or no, pulp reaction was shown. When the speed increased to 3,000-6,000 rpm, the reactions of the pulp when drilling without water was more obvious than when drilling with water. A significant increase in pulp reaction was observed when the speed was increased to 100,000-135,000 rpm with wet preparation and more pulp reaction was observed with a dry preparation. This result was in contrast to the result of Zach and Cohen (55) who found that cutting the tooth at a speed of 150,000 rpm produced less pulpal reaction than cutting at 20,000 rpm. This could be due to the different engines used in the experiments.

When considering the effect of vibration of the cutting instruments, the teeth prepared by rotary cutting instruments show no reaction of the pulp, whereas the teeth prepared by ultrasonic method show severe pulp changes (56). The lower of the speeds of handpiece, the more vibration is produced during preparation. Walsh and Symmons (57) demonstrated that speeds of 40,000 to 70,000 rpm, which produced by air turbine did not cause vibration and heat during preparation, therefore pulpal irritation might not occur. Seltzer and Bender (58) concluded that less pulpal reaction was found when preparing the tooth at the speeds of 50,000-150,000 rpm than did at the speeds of 5,000-20,000 rpm.

The reactions in the pulp also depend on the amount of friction and pressure, which cause from either low speed and high pressure or high speed and low pressure. Pressure applied on the tooth affects the vascular and lymphatic supply in the dental pulp, as a result, nutrients from outside cannot be transported into cells (59,60). The odontoblast cells are also aspirated to the dentinal tubules when applying pressure under the area that the extracting forceps contact the tooth. Seltzer (61) found that the pressure applied during full crown preparation with a low-speed handpiece caused severe damage to the pulps in dog teeth. The histologic evidence showed more severity when heat from the hot modeling compound was applied. He concluded that the combination of pressure and heat applied on the dental pulp could be extremely damaging.

Stanley and Swerdlow (50) demonstrated that the pressure applied on the tooth during preparation was more critical than the water coolant. The increasing pressure causes rising of the pulpal temperatures and damages the underlying odontoblast cells. They concluded that, if the applied forces were greater than 8 ounces, despite the control of frictional heat, a prominent inflammatory response could be initiated. Although the water coolant had the ability to reduce heat and prevent burn lesions, the drilling force greater than 8 ounces could not minimize inflammatory responses.

From the above, it can be concluded that the full crown preparation should be done with light pressure, and sufficient water spray coolant by using a good quality handpiece to reduce pulpal inflammation after preparation.

2.13.2 The remaining dentine thickness

Dentine is a good barrier that can protect the dental pulp from external irritants. When dentine is thick, the dentinal tubules are long, resulting in reduction of the concentration of external stimuli from outside the pulp. The external stimuli that can cause injury to the dental pulp are materials used in each process, cement from temporary or permanent cements or even bacteria from microleakage of restorations. The thick dentine can also reduce heat generated into the pulp during drilling the tooth surface. In tooth preparation procedure, especially crown and bridge abutment preparation, the dentine is cut to prepare the space of the restorative materials, as a result, the remaining dentine thickness is thin and the pulp can be injured. Stanley (62) suggested that the remaining dentine thickness of 2 mm would protect the pulp from most restorative materials and dental procedures. Recently, the estimated value of the minimal remaining dentine thickness which does not cause pulpal injury has been decreasing. The minimum dentine thickness that can prevent pulpal injury from the restorative materials from the study of Murray and colleagues (63) is only 0.5 mm. They studied the human pulp response by measuring the odontoblast cell numbers after preparing class V cavities with different remaining dentine thickness and filled the cavities with 3 types of material. They found that the number of odontoblasts beneath the cavity preparations was highly correlated to the remaining dentine thickness. When the thickness of the dentine reduced, the number of odontoblasts decreased. They concluded that the remaining dentine thickness of 0.5 mm or greater was necessary to

avoid evidence of pulpal injury. This study was consistent to the study of About and colleagues (64) who reported that the pulpal inflammation increased when the remaining dentine thickness decreased. They found that when the remaining dentine thickness was more than 1.1 mm, the only little inflammatory reaction was observed. If the bacteria were presented on the prepared surface, the inflammation increased significantly. In most cases in their experiment, the presence of bacteria is mainly attributed to microleakage.

Besides the remaining dentine thickness, the dentine permeability is one of the important factors which can protect the pulp from injury. Although the remaining dentine thickness between patients is similar, dentine permeability and pulp reactions may be different because the permeability is varied with patient age and treatment history (16).

2.13.3 The microleakage of the restorations

The acceptable marginal gap of crown and bridge restoration is ranged between 25-40 microns (ADA specification No.8). This gap is seal by dental cement to prevent bacterial leakage into the pulp. The temporary cement, which is used for bonding the temporary crown or bridge during fabricating the permanent restorations, is easy to dissolve in oral fluid. When the temporary cement is disintegrated, bacteria are able to invade into the dental pulp via the dentinal tubules, as a result, the dental pulp could be injured. After tooth reduction for crown and bridge, the pulp was irritated from preparation procedures. Bergenholtz and colleagues (65) suggested that the important thing to promote pulpal recovery was to prevent leakage of bacteria and bacterial byproducts.

The marginal leakage in fixed restoration has been always found in the provisional crowns and bridges due to the opened marginal gap. Lewinstein and colleagues (66) found that the marginal leakage of the provisional crowns was observed after temporary cementation for six days. Although the marginal gap of the permanent restorations was less than of the provisional restorations, the microleakage was still observed. Goldman and colleagues (35)found that there was marginal leakage in all three types of crown margin preparation; chamfer, shoulder, and shoulder with bevel. The leakage followed the dentinal tubules directly into the pulp, and the pattern was the

same in all types of preparation. They concluded that the microleakage was the important causative factor in pulp inflammation and possible pulp death.

To prevent pulpal inflammation from bacterial microleakage, the margin of the provisional restorations should be fit properly and the temporary cement should have the ability to seal the marginal gap. Furthermore, the provisional restorations should not be used for long period.

From the study of About and colleagues (64), they found that zinc oxide eugenol, used as a temporary cement, had the ability to prevent bacterial microleakage in 100 percent of cavity restoration for up to one year. This was due to an antibacterial activity and biologic sealing on the dentin surface of eugenol. On the other hand, zinc oxide eugenol is water soluble and has low strength so that it should not be used for long term.

2.14 Pulpal reactions to gingival retraction

Gingival retraction is one of many fixed prosthodontics procedures that can affect dental pulp response. Gingival retraction cord, which contains epinephrine directly effects on gingival blood flow and systemic blood pressure, whereas the retraction cord, which contains aluminum chloride does not (67). Epinephrine stimulates myocardial function, which increases the strength ventricular contraction and increases heart rate (68). It also acts as a vasoconstrictor, which affects many vascular systems. These actions result in an increase in blood pressure. Epinephrine in the retraction cord causes a significant decrease in gingival blood flow (67). There was no evidence that studied the effect of epinephrine in the retraction cord on blood flow in dental pulp. Banthitkhunanon and colleagues (69) recorded blood flow in the dental pulp before and after insertion of 5% aluminum chloride-impregnated retraction cords into the gingival sulcus using laser Doppler flow meter. They found that there was a significant reduction of blood flow signals during insertion of gingival retraction cord in mandibular second premolars and second molars. They claimed that the reduction of pulpal blood flow was due to the compression of the gingival blood vessels, resulting in a reduction of the light transmitted from the gingiva and total blood flow signal. Aluminum chloride did not effect on pulpal blood flow when applied to the gingival sulcus.

2.15 Dental cement

Dental cement is used to bond fixed restorations to abutment teeth and seal the marginal gap of the restorations. An ideal dental cement provides a proper adhesion between two materials (70,71). The ideal mechanical properties of the dental cement are high compressive and tensile strength, high fracture toughness, good wettability, adequate film thickness and viscosity to ensure complete seating, resistant to disintegration in the oral cavity to prevent bacterial microleakage, tissue compatible, and adequate working and setting times.

There are 2 types of dental cement; permanent cement used to bond permanent restoration, and temporary cement used to bond provisional or permanent restoration in a short duration (70). Permanent cement can be classified into water based and resin based cement. Water based cements comprise zinc phosphate cement, polycarboxylate cement and glass ionomer cement. Resin based cements are resin modified glass ionomer cement and resin cement.

Temporary cement can be classified into resin based and oil based cement (70). Resin based cement is temporary resin cement. Oil based temporary cement are zinc oxide eugenol (ZOE) and zinc oxide noneugenol cement (ZONE). Zinc oxide eugenol cement has a sedative effect on exposed dentine and it is useful on exposed dentinal tubule. The important properties of temporary cement, follow ANSI-ADA spec No. 30, are that it should have moderate strength and low acidic property. The pH of ZOE is neutral so they do not require protective varnish or liner prior to cementation.

There are 4 types of zinc oxide eugenol cement (70). Type I is temporary cement, type II is permanent cement which is polymer reinforced ZOE, type III are filling material and bases and type IV is cavity liner. The powder of type I ZOE, which is temporary cement comprise 69% zinc oxide with added 29% rosin to reduce the brittleness of the set cement, zinc stearate as a plasticizer, and zinc acetate to improve the strength of the cement. The liquid is eugenol with olive oil as a plasticizer. Type II ZOE, used as permanent cement, comprises zinc oxide 80%, acrylic resin 20% and alumina. Orthoethoxybenzoic acid (EBA) is added into the liquid, which gives cement stronger than type I.

The setting reaction of ZOE is chelation reaction (70). Water is needed to initiate the reaction result zinc hydroxide and then react with eugenol to form zinc eugenolate and by-product is water.

$$ZnO + H_2O \rightarrow Zn(OH)_2 + \text{eugenol} \rightarrow \text{zinc eugenolate} + H_2O$$

The accelerator of the reaction is humid or water and zinc acetate dehydrate which is more soluble than zinc hydroxide and can supply zinc ions more rapidly. Another accelerator is an acetic acid which is more active catalyst than water. High atmospheric pressure also accelerates the reaction.

The effects of eugenol depend on its concentration in tissue (72). Eugenol concentration in zinc oxide eugenol cement is about 10^{-2}mol/l, which is cytotoxic for bacteria. From the study of Abou Hashieh (73), eugenol was not cytotoxic at low concentrations (from 10^{-9} to 10^{-6}mol/l). However, it started to be cytotoxic at 10^{-5}mol/l and was lethal to 95% of the cells at 10^{-3}mol/l.

From the reaction above, there is no excess of eugenol because all of eugenol is reacted with the excess zinc oxide, which has more amount than eugenol. Eugenol is the product of hydrolysis of zinc eugenolate so that there is no eugenol diffused from ZOE until exposed to water. This means that the wetness of the local environment determines how much eugenol is released, and how rapidly. It was found that the amount released is proportional to the exposed surface area, but not to the thickness of ZOE (4). Eugenolis slowly released and it flows slowly inward to the pulp. It is harmful to the pulp when applied directly on the exposed thin dentine, especially on wet dentine, and may cause pulpal necrosis. When the remaining dentine is thin, eugenol diffusion from temporary cement may reach a cytotoxic threshold in the pulpal fluid. Since eugenol is released from zinc oxide eugenol cement by hydrolysis, the fluid from the dentinal tubules can cause more diffusion of eugenol. Moreover, if there is marginal leakage of the restorations, water from oral cavity could liberate more eugenol. Thus, more eugenol would be released from poorly sealed restorations than in a well-sealed restoration. Another factor that affects pulp and dentine response is the cavity depth. When remaining dentine thickness is less than 0.5 mm, it has high permeability so that eugenol releases more quickly than with thick dentine (73). Significantly, the deeper the

preparation, the greater the effect on the odontoblasts and subsequent dentine formation. This would indicate that preparation close to the pulp had a suppressive rather than stimulatory effect on the odontoblasts (74).

The permeability of dentine is related to the number and diameter of the dentinal tubules (31). Therefore, it is possible that in deep cavity preparations, bacteria can migrate through the remaining dentine into the pulp. The important biological properties of eugenol are an antimicrobial activity (75-77), anti-inflammatory property (77-79), and bactericidal and analgesic effects (75). Eugenol increases the antimicrobial functions of neutrophils, which are the important factor in host defense mechanism against invading microorganisms (80). The mechanism in anti-inflammatory action of eugenol is by inhibition of PGE_2 and interleukin 1β synthesis (77). The mechanism of analgesic effect is by inhibition of calcium ion channel (75). When used in low concentration, eugenol is slowly released to dentine and reduces dentine sensitivity and pulpal inflammation from tooth preparation procedure (72). It also inhibits nerve activity and white blood cell chemotaxis. The antimicrobial activity of eugenol might cause from a tight seal of the cement, which could prevent bacterial microleakage into the pulp (81,82).

On the other hand, eugenol has markedly toxic effects when applied directly to the soft tissues including dental pulp cells, gingival fibroblasts and periodontal ligament fibroblasts (78,83,84). It can irritate the pulp when applied directly on the exposed thin remaining dentine. When dentine thickness is less than 0.5 mm, eugenol released faster than with thick dentine (4,5). Brannstrom and colleagues (82)found that zinc oxide eugenol cement caused an inflammatory reaction in the pulp where the distance to the pulp was less than 0.5 mm.

The cytotoxic and genetic effects of eugenol can be associated with oxidative DNA damage by its metabolites (85). Moreover, eugenol suppressed the synthesis of type I-V collagens, the expression of mRNA and protein, suggesting that eugenol may affect both syntheses of collagenous and non-collagenous proteins.

The important adverse effect of eugenol is that it inhibits resin polymerization. Fujisawa and Kadoma (6)reported that eugenol is phenol derivative. Benzoyl peroxide,

an indicator in resin cement, decomposes very rapidly in the presence of phenol derivative. It acts as a retarder against the polymerization of the resin cement. Bayindir (86) reported that eugenol is chain-breaking of resin polymerization. He concluded that temporary cement with eugenol was significantly reduced the bond strength of full crown casting with resin cement.

Terata and colleagues (87) found that eugenol decreases transverse bond strength, surface hardness and shear bond strength and increases surface roughness and surface discoloration. Moreover, it inhibits resin polymerization. The residue of ZOE also alters the bonding properties of polycarboxylate, glass ionomer and resin cements, resulting in impairment of the bond strength of those cements (88,89). This effect also happens in the bond between composite resin and tooth structure. Eugenol can alter the surface of cured resin which significantly decreases bond strength between composite and dentine (5,90). If eugenol directly contacts before curing the composite resin, they do not achieve a full cure or hardness (91). This is probably due to the ability of eugenol to penetrate the tooth surfaces (73). As a result, it may have changed the characteristic of tooth structure such as contact angle and dentine permeability (92).

From the negative effect mentioned above, various types of carboxylic acids are used to replace eugenol to produce a ZOE-like material, which is called zinc oxide-noneugenol cement. This type of cement is used when the final restorations are planned to be fixed with resin cement. Woody and Davis found that the negative effect may not be caused by eugenol but by the presence of residual cement (93). Bayindir and colleagues found that the residual cement reduced the tensile bond strength of resin cement, and ZOE effects more than ZONE (86). If the residual cement cannot be removed carefully, the bond strength between dentine and adhesive cement can be significantly decreased. However, Mayer and colleagues (94) reported that lower bond strengths were related to the eugenol effects, not to cement residues. From the effects of eugenol and residual cement in temporary cement, dentine surface should be cleaned prior to permanent cementation to improve the bond strength of tooth structure and permanent cement.

When resin cement was planned to be used as permanent cement, total etch technique, performed by applying acid etching on exposed enamel and dentine, demineralized hard tissues to prepare tooth surface to be bonded with dental adhesive material. The collagen in the intertubular matrix of the dentine is exposed after acid etching. The peritubular dentine demineralizes faster than does the intertubular matrix. This demineralization widens the tubules allow theresin to penetrate to increase bond strength to dentine. The demineralization should not denature the collagen because the collagen forms an interwoven mesh of fibers that the resin will infiltrate (Fig. 2.5). This collagen mesh infiltrated by resin is referred as the hybrid layer, which is about 5-10 μm thick. The wetness of the hybrid layer is very important. If the hybrid layer is too dry, the collagen mesh will collapse and resin will not be able to penetrate into the mesh.

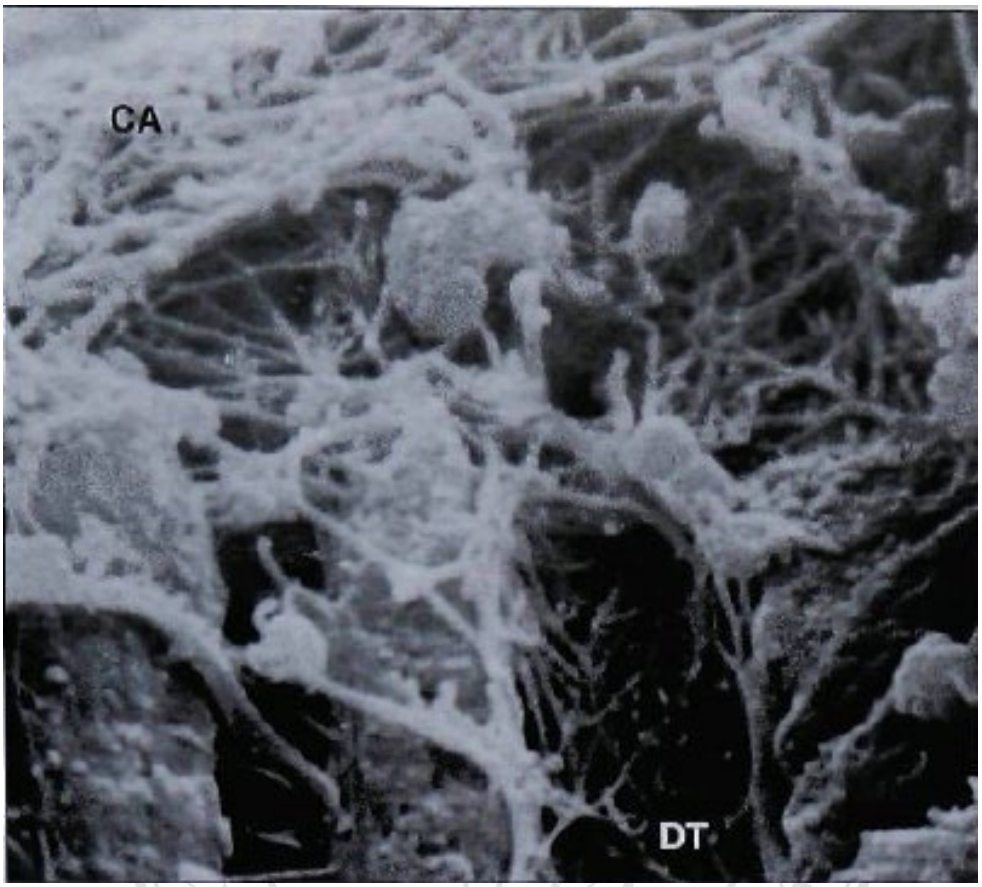

Figure 2.5 Scanning electron micrograph of prepared and acid etched dentine. It shows the inter woven fibers on the cavity (CA) floor and in the wall of the dentinal tubule (DT) (39).

2.16 Laser Doppler flowmetry

The one important information for diagnosis and treatment planning in dental work is the vitality of the tooth. There are several methods to obtain this information, including thermal testing and electric pulp testing. However, these tests imply nerve response, not blood circulation. The tests which stimulate nerve fibers are not the ideal methods of vitality test (95). The vitality of the dental pulp indicates that there is blood circulation within the tissues. Thus, the only test that can be called a vitality test is the test that measures pulpal blood flow, which is the most accurate determinant in assessing the vitality of the pulp. As a result, teeth that have temporarily or permanently lost their sensory function may be non-responsive to these tests.

Laser Doppler flow meter was introduced in dentistry in the late 1980s (7,21). It is used to detect the blood supply of the pulp. It is an optical, noninvasive, painless and semiquantitative measuring instrument. It evaluates dynamic changes in blood flow by detecting blood cell movement in a small volume of tissue (7). Laser light is transmitted to the dental pulp by means of a fiber optic probe, which placed on the tooth surface. Two equal-intensity beams intersect across the target area. Red blood cells moving within the volume, illuminated by the beam, will cause the light to change frequency. This change in frequency is called a Doppler shift (Fig. 2.6). The scattered light beams from the static tissue will not change the frequency. The reflected light, composed of Doppler-shifted and unshifted light, is returned to the same probe via an afferent fiber to a photodetector in the flow meter and an electrical signal is produced (96). The photodetectors convert the mixing of shifted and unshifted light into a semiquantitative measurement, called the flux signal of blood flow. Flux or output signal is the number of moving red blood cells per second times their mean velocities (95). The readout of flux signal is in perfusion units (P.U.). It is a relative value expressed in arbitrary measurement units. No current laser Doppler device can present absolute perfusion values of blood flow, so the P.U. from the different type of devices cannot be comparable, and even for the same device they could vary at different times.

To assess the vitality of teeth, the flux signal from a vital tooth can be compared with that of nonvital tooth. The flux signal from a vital tooth should be greater than from a nonvital tooth due to differences in blood flow (97). Some authors (98) reported that Laser Doppler flow meter can distinguish teeth with necrotic pulp from teeth with vital pulp, but others (96,97,99) showed that this instrument is not a reliable method for assessing pulpal vitality. They found that the signal recorded from a tooth is not only derived from the dental pulp, but also from periodontal tissues.

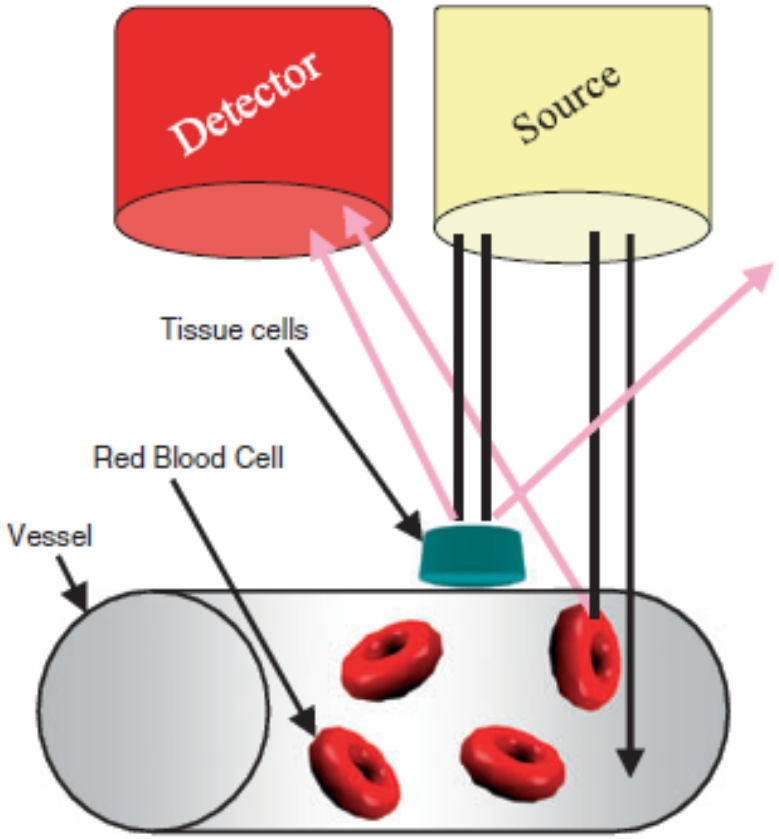

Figure 2.6 Diagrammatical illustration of Doppler principle (100).

Although it has been shown that the output signal from a nonvital tooth is significantly lower than that from a vital tooth, the values recorded from most nonvital teeth have never been zero (101,102). Nonvital teeth should not be used as controls in comparing blood circulation in the pulp because the optical properties of pulp and dentine will change after pulp died, and such changes will affect the amount of light transmitted to tissues outside the tooth. The study of Soo-ampon and colleagues (97) showed that the record from a tooth which the pulp had been removed and replaced was different from that obtained from the same tooth when the pulp chamber was left empty. Although the pulp is death, no circulation of red blood cells, the recorded signal is different from that of the empty pulp which also has no movement of red blood cells. This is due to the effect of the pulp tissue on light transmission.

There are many factors influencing the signal blood flow, such as probe design, probe holder characteristics, gingival isolation, flowmeter characteristics, mineralization of enamel and dentine, the temperature of the environment, the position and the resting status of the patient, the position of the probe, heartbeat-synchronous oscillations, tooth discoloration, stress, intake of drugs, age-related changes, etc (100).

The main problem in this instrument is to measure blood flow through mineralized tissues that limit the penetration of the laser beam into the tooth. However, it has also been reported that the ability of these instruments to detect blood flow may be due to the dentinal tubules acting as light guides (8). Thus laser light applied to teeth can reach the periodontal tissues through tubular dentine and return to the probe. As a result, the total signal from this device may not obtain only from the dental pulp (26,103).

Nonpulpal signals can contaminate the flux signal recorded from the pulp. These signals may be derived from periodontal tissues, lips, tongue, labial and palatal gingiva. The study of Soo-ampon and colleagues (97) showed that only approximately 43% of the blood flow signal recorded from an intact tooth with rubber dam is due to blood flow in the pulp. Vongsavan and Matthews (104) reported that about 15% of the signal recorded with the laser probe centered 2 mm from the gingival margin was of non-pulpal origin. These results contrast with those of the other studies which suggest that up to 80% of the signal recorded from human teeth may be of non-pulpal origin. This discrepancy might be due to species differences.

Many studies suggest to measure pulpal blood flow with a rubber dam to isolate the crown because they found that the signal recorded from an intact tooth in human is reduced by approximately 80% when the gingival and surrounding tissues are covered with a black rubber dam (26,96,97,105). This reduction can be effected by rubber dam for two reasons (97). First, the dam screened the light from periodontal, gingival and other surrounding tissues. Second, the compression of gingival tissue from the dam reduced gingival blood flow. Some authors suggested to apply cotton rolls (26) and periodontal paste (103) to reduce the signal from neighboring tissues. However, the stability of the laser probe is also important. Any movement of the probe can cause artifacts of signal blood flow. Hartmann and colleagues (26) suggested that the probe

should be supported by rigid or solid splint rather than silicone biteblock. They found that a rigid plastic splint used with rubber dam gave more satisfactory results than a silicone biteblock.

One of the factors that can affect pulpal blood flow signals is the size of the dental pulp. Although the crowns have the same size, the size of the pulp may not similar. The pig teeth in the study of Vongsavan and Matthews (104) were similar in size to human incisors but the pulps were much larger. The pulpal blood flow signal derived from the pulp in pig teeth was greater than from human pulp. This may also due to the difference in species, resulting in the different optical properties of dentine and enamel.

The wavelength of the light also affects the recorded signal. The light with long wavelength such as infrared penetrates tissues to a greater depth than shorter wavelength such as red light. Kijsamanith and colleagues (106) found that blood flow signal recorded with infrared light from the human teeth covered with rubber dam reduced greater than that recorded with red light. The reduction was 82% with infrared and 56% with red light. Without rubber dam, the infrared light produced higher signal values than red light. This was due to the deeper penetration of the infrared light into the surrounding tissues. After applying the rubber dam, the light that transmitted to the surrounding tissues was blocked by rubber dam, as a result, the blood flow signal reduced significantly.

With the limitations of this instrument, the method of recording pulpal blood flow should be performed carefully, and the data should be interpreted with care.