CHAPTER II

LITERATURE REVIEW

Surgical root canal therapy, including root-end resection, has been practiced since at least the mid-1800s. In 1906 Schamberg described using radiographs to assist diagnosis and the use of surgical burs to perform a rapid osteotomy and root-end resection (Johnson and Witherspoon 2006). Since 1930s, most of the surgical treatment involved some type of cutting away of a portion of the root. The reason was revealed later that approximately 75% of the teeth have canal aberrations in the apical 3 mm of the tooth (De Deus 1975;Seltzer et al. 1966). An apical resection of approximately 3 mm should include most accessory and lateral canals. However, the apical foramen should be sealed surgically from the apex into the tooth and this procedure has been referred to as a reverse filling or retrofilling.

The requirements of an ideal retrofilling material are that the material should: (Gartner and Dorn 1992;Kim 2001)

1. adhere or bond to tooth tissue and seal the root end three dimensionally
2. not promote, and preferably inhibit, the growth of pathogenic microorganism
3. be well tolerated by periradicular tissues with no inflammatory reaction
4. stimulate the regeneration of normal periodontium
5. be non toxic both locally and systemically

6. be dimensionally stable and unaffected by moisture in either the set or unset state
7. not corrode or be electrochemically active
8. not stain the tooth or the periradicular tissues
9. be easily distinguishable on radiographs
10. have a long shelf life
11. be easy to handle or manipulate
12. be not expensive

Many materials have been suggested as retrofilling materials including gutta percha, amalgam, polycarboxylate cement, zinc phosphate cement, IRM, EBA, Cavit, glass ionomer, resin composite, MTA, gold foil, cyanoacrylate, Diaket, Titanium screw, Teflon (Torabinejad and Pitt Ford 1996). However, only some materials have been commonly used in clinical practice within the past 10 years. These materials are zinc oxide-eugenol cement (IRM and superEBA), glass ionomer cement, resin composite, resin glass ionomer hybrids and mineral trioxide aggregate (MTA). However, no material that fulfills all or most of the ideal properties of a retrofilling material has been found.

1. Mineral Trioxide Aggregate (MTA)

Mineral Trioxide Aggregate (MTA) was developed as a new retrofilling material at Loma Linda University, California, USA in 1990.

The MTA powder consists of fine hydrophilic particles. The main constituents of this material are calcium silicate, bismuth oxide, calcium carbonate, calcium sulfate, and calcium aluminate. When mixed with sterile water, hydration of the MTA powder results in a colloidal gel that solidifies into

a hard structure consisting of discrete crystals in an amorphous matrix. The crystals are composed of calcium oxide and the amorphous matrix which has the composition of 33% calcium, 49% phosphate, 2% carbon, 3% chloride, and 6% silica (Torabinejad et al. 1995b).

Physical and chemical properties of MTA has been investigated in a study which pH, radiopacity, setting time, compressive strength and solubility of the material were compared with amalgam, superEBA and IRM (Torabinejad et al. 1995b). The pH of MTA rises from 10.2 after mixing to 12.5 after 3 hours. The pH has been reported to be approximately 9.5 at 168 hours after mixing (Duarte et al. 2003). For radiopacity, MTA was found to be less radiopaque than amalgam but more radiopaque than superEBA and IRM. MTA had the longest setting time which was 2 hours and 45 minutes. However, MTA had the lowest compressive strength at 24 hours after mixing which was 40.0$\pm$4.4 MPa and the compressive strength increased to 67.3$\pm$6.6 MPa at 72 hours after mixing. The solubility of MTA after setting was similar to that of amalgam and superEBA. When compared solubility of MTA at 1, 7 and 21 days, MTA showed low solubility and there was no difference between time period (Torabinejad et al. 1995b).

The sealing ability of MTA was investigated using fluorescent dye and confocal microscopy (Torabinejad et al. 1993), methylene blue dye (Torabinejad et al. 1994) and bacterial marker (Torabinejad et al. 1995d). Its marginal adaptation was assessed using electron microscopy (Torabinejad et al. 1995e). The long term seal was measured over a 12-week (Bates et al. 1996) and 12-month period (Wu et al. 1998) using different fluid filtration

methods. They all reported good results with MTA when ranked with other materials which mostly were amalgam, IRM and superEBA.

Biological property of MTA was also investigated in biocompatibility and cytotoxicity aspects. The biocompatibility assessment of MTA encompassed *in vitro* cell culture techniques using either established cell lines or primary cell cultures. The results showed MTA to be biocompatible (Balto 2004a;Bonson et al. 2004;Keiser et al. 2000b;Koh et al. 1997;Koh et al. 1998; Osorio et al. 1998). *In vivo* usage testing revealed less periradicular inflammation with MTA compared with amalgam (Torabinejad et al. 1995a;Torabinejad et al. 1997).

Besides, MTA has the ability to encourage hard tissue deposition. There was an *in vivo* study that showed a complete layer of cementum over MTA retrofilling (Torabinejad et al. 1997). With implantation of MTA in the tibias and mandibles of guinea pigs, the most favorable tissue reaction was observed at both sites. In tibia, MTA was the material most often observed with direct bone apposition when compared to amalgam, IRM or superEBA (Torabinejad et al. 1998).

2. Zinc oxide-eugenol cements

Zinc oxide-eugenol cements have been recommended for retrofillings by clinicians for many decades (Nicholls 1965). IRM and superEBA are 2 examples of such materials. SuperEBA was adopted to improve the physical properties of zinc oxide-eugenol cements by partial substitution of eugenol liquid with ethoxybenzoic acid (EBA) and addition of fused quartz or aluminum oxide to the powder. The use of superEBA as a retrofilling material was

suggested by Oynick and Oynick (1978). SuperEBA consists of a powder containing 60% zinc oxide, 34% aluminum oxide, and 6% natural resin. It is mixed in equal parts with a liquid that contains 37.5% eugenol and 62.5% ethoxybenzoic acid. SuperEBA is available in two forms: fast set and regular set. Apart from setting time, the sealing ability of the two forms appear to be the same (Yaccino et al. 1999). SuperEBA has radiopacity and sealing effects similar to those of IRM but is less leaky than amalgam (O'Connor et al. 1995;Shah et al. 1996). However the acid environment as in the periradicular wound, superEBA was shown to be disintegrated over time which may affect the long-term stability (Arnold et al. 1997).

SuperEBA induces mild to moderate toxicity when freshly mixed. The toxicity of superEBA is probably due to eugenol. Cytotoxicity diminishes rapidly as the cements set and long term inflammatory potential appears to be minimal (Pitt Ford et al. 1995). In an implantation experiment, there was a slightly greater inflammatory response with superEBA than amalgam or IRM but by 100 days all three material showed complete healing (Olsen et al. 1994). In a clinical study, superEBA showed similar results of bone healing as compared to amalgam (Pantschev et al. 1994).

Although superEBA provides an optimum seal and minimal tissue toxicity, it is difficult to mix. It requires more effort and practice than any other retrofilling materials. Another problem is that its radiopacity is similar to that of gutta percha. Ideally, the retrofilling material should be readily distinguishable from tooth structure and the obturating material.

3. Resin composites

Resin composite is a blend of aromatic and/or aliphatic dimethacrylate monomer such as bis-GMA, triethylglycol dimethacrylate (TEGDMA) and urethane dimethacrylate (UDMA). Resin composite materials have some desirable properties and may be considered for use as retrofilling materials. From systematic reviews of *in vitro* leakage of retrofilling materials, the results indicated that the most effective retrofilling material when measured by dye/ink penetration is resin composite which is more effective than glass ionomer cement, amalgam, and superEBA (Theodosopoulou and Niederman 2005). While *in vivo* review of effectiveness determined by reduction in periapical radiolucency, the results showed that resin composite and superEBA were more effective than amalgam (Niederman and Theodosopoulou 2003). However, certain components of resin composite and dentin bonding agents can have a cytotoxicity effect on cells (Bruce et al. 1993;Hanks et al. 1992;Rakich et al. 1998;Vahid et al. 2004). Some studies have shown that once the resin composite set, cells could adhere and spread on its surface (Peltola et al. 1992;Zhu et al. 2000).

Flowable resin composite has been suggested to use as retrofilling material because of its desirable properties of ease in placement. An SEM photomicrographs from a study of flowable resin composite used as retrofilling showed that there was no interface gap formation when used in combination with dentin bonding agent (Tam and Yu 2002). There was no inflammatory cell infiltration detected in flowable resin composite group used in cervical cavity when evaluating the pulpal response (Shimada et al. 2004).

Retroplast is a chemically cured flowable resin composite developed in 1984 specifically for using as a retrofilling material. Retroplast is a two paste system. Paste A is composed of Bis-GMA/TEGDMA 1:1, benzoyl peroxide N, N-di-(2-hydroxyethyl)-p-toluidine. This is mixed in equal parts with paste B, which is composed of resin ytterbium trifluoride, ferric oxide. A Gluma-based dentin bonding agent is used to adhere the material to root-end surface. The working time is 1 ½ to 2 minutes, and the radiopacity (due to the ytterbium trifluoride) is equivalent to 6 mm of aluminum.

Retroplast used for retrofilling in a saucer-type preparation has achieved good short-term and long-term healing results in clinical studies (Rud et al. 1991a;Rud et al. 1991b;Rud et al. 1996). Clinical investigation including patient recalls of up to 9 and 12 years after treatment with Retroplast showed complete radiographic bone healing (Rud et al. 2001). *In vivo* studies in both monkeys and humans revealed the absence of inflammatory cells around the retrofilling with fibroblast and collagen fibers present immediately adjacent to the filling (Rud et al. 1991a). It is also shown *in vivo* that cementum deposition with sharpey's fiber was found in intimate contact with the restoration, indicating that tissue regeneration including cementogenesis may occur on composite material. However in cases where there was poor hemostasis during surgery, there was an absence of complete healing, possibly because of bond failure between the Retroplast and root dentin (Rud et al. 1991b). Therefore, maintaining a dry field is important when using Retroplast.

Eventhough there are many studies have shown remarkable clinical success of Retroplast, unfortunately Retroplast is not yet available in Thailand.

Therefore other available flowable resin composite might be considered as candidates for this procedure.

Tetric® Flow (Ivoclar Vivadent, Liechtenstein), Filtek™ Flow flowable resin composite, manufactured by 3M ESPE (USA) and Aeliteflo™ (Bisco, USA) are three flowable resin composites widely available in clinical practices. All of them are low viscosity, visible-light curing, radiopaque fine-particle hybrid composite for the restorative therapy. Filtek™ Flow and Aeliteflo™'s monomer matrix contain BisGMA and TEGDMA while Tetric® Flow's contains Bis-GMA, UDMA, TEGDMA. Due to their flow ability and availability, these three flowable resin composites will be tested in this study.

Cytotoxicity testing

In vitro cytotoxicity screening methods have been widely used to evaluate biocompatibility of material. There are many test systems available to determine the cytotoxicity of dental materials in cultured mammalian cell population. Morphological assays observed the changes at the cell surface or the cellular cytoskeleton. Functional assays typically evaluate the cell's capacity to provide energy sources necessary for anabolic activities, or the end products of such activities (Schweikl and Schmalz 1996).

Colorimetric (MTT) assay is one of the functional assays that use tetrazolium salt MTT ((3-(4, 5,dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to measure mitochondrial dehydrogenase activity. It is a pale yellow substrate that produces a dark blue formazan product when cleaved by active mitochondria, and so the reaction only occurs in living, metabolically active cells (Mosmann 1983). Therefore this assay can indicated the effect of dental

materials on cell viability by alterations of mitochondrial dehydrogenase activities and the amount of formazan generated was directly proportional to the cell number. It was also shown earlier that activated cells produced more formazan than resting cells. This assay performed very effectively and only a few cells are needed for rapid, reliable and inexpensive screening purposes of a large number of samples in a short time (Schweikl and Schmalz 1996).

Scanning electron microscope was widely used for morphological assay in order to observe the changes in cell morphology, adhesion and spreading on dental material. Adhesion and spreading of the cells on a materials surface are the initial phase of cellular function. Rajaraman et al (1974) examined the adhesion and spreading of fibroblasts cells in cultured and found discovered that prior to adhesion, cells were spherical or ovoid in shape and were covered with surface blebs and/or short microvilli. Adhesion was initiated by contact of the microvilli with the substratum. This was followed by formation of long filopodia at the point of contact and a decreased number of surface blebs and/or microvilli on cell surfaces that were not in contact with substratum. Cell spreading then occurred, with peripheral expansion of cytoplasmatic webs (lamellipodia) composed of many filopodia. After that, flattening of the cells into a polygonal shape appeared with decreasing in number of microvilli and filopodia (Rajaraman 1974). The persistence of rounded cells with little or no spreading would suggest the surface material may be toxic (Zhu et al. 2000). Balto (2004b) described the cytoplastmic surfaces extensions formed by cells after incubated with root-end filling materials in terminology as described. Lamellipodia is the flattened extensions usually approximately 0.1-0.5 μm thick. Filopodia is a cylindrical or conical process of smaller diameter. It is often up to 10-20 μm long. Microvilli is the

process of smallest diameter usually 0.1-0.2 μm. And blebs are spherical or hemispherical in shape and usually 1 to 2 μm in diameter which referred to shrinkage of cytoplasm. This may cause breaks of the cell membranes resulting in excretion of cell content including organelles.

Periradicular wound healing

The ideal healing response after periradicular surgery is the re-establishment of an apical attachment apparatus including cementum overlying the resected root-end surface, periodontal ligament and osseous repair (Andreasen 1973;Craig and Harrison 1993). However, histological examination of biopsy specimens revealed three types of tissue response: healing with reformation of periodontal ligament; healing with fibrous tissue (scar); and moderate-to-severe inflammation without scar tissue (Andreasen and Rud 1972). The deposition of cementum on the cut root surface is considered a desired healing response and a prerequisite for the reformation of a functional periodontal attachment.

Human periodontal ligament cell contains heterogeneous cell populations that can differentiate into either cementum-forming cell (cementoblasts) or bone-forming cell (osteoblasts) (Lekic et al. 2001;Murakami et al. 2003). Periodontal ligament stem cells also have capacity to generate a cementum/periodontal ligament like structure when transplanted into *in vivo* (Seo et al. 2004). PDL cells have many osteoblast like properties including the capacity to express the bone-associated markers alkaline phosphatase (Wlodarski and Reddi 1986). It has been shown that the alkaline phosphatase measurement is the most reliable marker of cell osteoblastic phenotypes (Hoang et al. 1997;Nojima et al. 1990;Piche et al. 1989).

Studies that used established cell line have the advantage of enhanced reproducibility of results and are recommended by the ISO for preliminary cytotoxicity screening. For specific sensitivity testing to stimulate the *in vivo* situation, primary cell strains derived from living tissue are necessary and are also recommended by the ISO (Keiser et al. 2000b).