CHAPTER I

INTRODUCTION

1.1 Background and rationale

The dental pulp may become exposed to the oral environment by many ways such as carious lesions that extend through the enamel or cementum and dentin, tooth fracture, or dental operative procedures (Mjör, 2002). When the pulp is mechanically exposed or superficially infected, it is beneficial to preserve the vitality and health of the exposed pulp rather than replacing it with a root canal filling material. The major reason is because the pulp is a well vascularized and innervated connective tissue that has self-healing capacity unless properly treated. Reparative dentin formation is an important defense mechanism that protects the pulp from noxious elements in the oral cavity (Kakehashi *et al.*, 1965; Cvek, 1978; Schröder, 1985; Yamamura, 1985; Murray *et al.*, 2002; Goldberg *et al.*, 2003). Until now, the major concern of endodontics has been the prevention or elimination of apical periodontitis. The conservative treatment of vital pulp is, therefore, the best way to ensure the prevention of periradicular pathology (Trope, 2003).

Vital pulp therapy may be broadly defined as any aspect of restorative dental treatment intended to minimize trauma to the dental pulp, including direct and indirect pulp capping, partial and complete pulpotomy (Rutherford and Fitzgerald, 1995). The objective of vital pulp therapy is to obtain healing of a pulpal wound in order to preserve

a vital tooth with a healthy pulp (Ward, 2002). The opened exposure has to be sealed off by using an appropriate wound dressing to prevent bacterial contamination and promote pulp healing. Following proper disinfection, debridement and the absence of microorganism, soft and hard-tissue healing have been shown to occur at a very high rate (Kakehashi *et al.*, 1965; Cvek, 1978; Baume and Holz, 1981; Mejàre and Cvek, 1993).

Calcium hydroxide has been introduced in capping the exposed pulps for many decades (Zander, 1939; Glass and Zander, 1949; Kozlov and Massler, 1960; Stanley and Lundy, 1972; Tronstad, 1974). Its high alkalinity provides the anti-bacterial property and encourages tissue repair. When it is applied to the exposed pulp, it cauterizes superficial tissue and causes the zone of coagulative necrosis. This firm necrotic layer irritates the underlying tissue and stimulate hard-tissue repair (Schröder and Granath, 1971). The formation of dentinal bridge has been believed to be the principal biological result for a successful treatment of an exposed or amputated pulp (Kozlov and Massler, 1960; Rowe, 1967; Stanley, 1989; Mjör *et al.*, 1991).

Although pulp capping with calcium hydroxide has been accepted to be highly successful, many disadvantages have been reported. The morphology of the hard-tissue bridge is often irregular, with cellular inclusions and tunnel defects. Thus, it often becomes highly permeable to bacteria and bacterial elements, and increases the risk of pulpal infection from possible surface seal breakdown (Cox *et al.*, 1996). The softening and disintegration phenomenon of calcium hydroxide has been demonstrated. Its

unstable physical properties may allow material particles to migrate into pulp tissue and cause inflammatory response (McComb, 1983; Hwas and Sandrik, 1984). Most calcium hydroxide medicaments have been reported to disintegrate and wash out after 6 months, leaving a void underneath the restoration and thereby a pathway for bacterial infection (Cox *et al.*, 1996). It is also disintegrated by phosphoric acid-etching agents (Phillips *et al.*, 1984) and allows long-term softening of the adjacent composite resin (Cox and Suzuki, 1994).

In order to overcome the previously described problems of direct pulp capping with calcium hydroxide, there have been various attempts to use other materials such as adhesive materials (Heitmann and Unterbrink, 1995; Olmez *et al.*, 1998; Tarim *et al.*, 1998; Kitasako *et al.*, 2002; Scarano *et al.*, 2003), hydroxyapatite (Jaber *et al.*, 1991; Subay and Asci, 1993), tricalcium phosphate (Chohayeb *et al.*, 1991) and mineral trioxide aggregate (Ford *et al.*, 1996; Tziafas *et al.*, 2002). The recent literatures have been interested in a number of biologic molecules as an alternative way to stimulate pulpal regeneration (Rutherford *et al.*, 1993; Rutherford and Fitzgerald, 1995; Goldberg *et al.*, 2003; Tziafas, 2004). Although the new effective capping materials have been introduced, calcium hydroxide has remained the gold standard as pulp capping material primarily due to solid clinical documentation (Haskell *et al.*, 1978; Baume and Holz, 1981; Fitzgerald and Heys, 1991).

Further experiments are needed to discover more effective materials that may provide clinicians with additional options for treatment of exposed vital pulps. The agent

used in vital pulp therapy should ideally be non-toxic, possess anti-microbial and anti-inflammatory activities in order to control pre-existing inflammatory states of the exposed pulp and operative-induced inflammation (Ward, 2002). The principal objective of capping procedure is to obtain pulp tissue healing. Therefore, an anti-inflammatory agent, such as corticosteroids, might be considered as a candidate for this purpose.

Topical corticosteroid is glucocorticoid hormone that uses topically in treatment of various dermatologic disorders such as itching, redness, dryness, crusting, scaling, inflammation, and discomfort of many skin conditions (Maibach and Stoughton, 1973; Sneddon, 1976; Pariser, 1991). They are also being used in various types of oral diseases: minor and major aphthous ulceration, herpes simplex, lichen planus, erythema multiforme, mucous membrane pemphigoid, epidermolysis bullosa, pemphigus vulgaris, bullous pemphigoid, dermatitis herpetiformis, lupus erythematosus (Kay, 1976).

Some advantages of topical corticosteroids have been reported such as anti-inflammatory action (Rapoport and Abramson, 1958; Ulmansky *et al.*, 1971; Fachin and Zaki, 1991), pain reduction or pain relief (Fry *et al.*, 1960; Schroeder and Triadan, 1962), do not inhibit dentin bridge formation (Schneider, 1968; Barker and Ehrmann, 1969; Barker *et al.*, 1972). However, possible adverse effects are also reported such as adrenal suppression, Cushing's syndrome, striae, allergic contact dermatitis (Maibach and Stoughton, 1973), inhibition of the cellular response to an irritant that may increase risk of infection and bacteremia (Sinkford and Harris, 1964; Klotz *et al.*, 1965),

suppression of fibroblastic mitosis (Taylor *et al.*, 1989), and collagen biosynthesis (Uitto *et al.*, 1972).

Topical glucocorticoids have been of interest by many researchers for pulpal treatment. Hydrocortisone acetate was the first glucocorticoid which successfully used in treatment of human vital pulp (Rapoport and Abramson, 1958). Triamcinolone acetonide is a major constituent in Ledermix®, which is the first corticosteroid-antibiotic agent used in vital pulp and endodontic therapy (Schroeder and Triadan, 1962; Barker and Ehrmann, 1969; Ulmansky *et al.*, 1971; Barker *et al.*, 1972; Paterson, 1981).

There was a case report that pulpal obliterations are observed in patients treated with long-term systemic corticosteroids. It was proposed that glucocorticoid therapy may induce excessive dentin formation (Symons and Symons, 1994). From a recent laboratory study; dexamethasone was able to stimulate osteogenic differentiation in human dental pulp cell cultures, strongly stimulate alkaline phosphatase activity, and induce the expression of dentin sialophosphoprotein (Alliot-Licht *et al.*, 2005).

Fluocinolone acetonide has provided distinct advantages in topical therapy. It has been frequently used as topical medicament in treatment of various oral vesibuloerosive lesions and available at the Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand. There is an evidence supported that it has transient stimulatory effect on cell proliferation and multiplication in cultured human skin fibroblasts (Kirk and Mittwoch, 1977). However, the effect on pulpal healing or repair process is unknown. The research interest is focused on fluocinolone acetonide whether

it could promote pulpal healing and could be developed as a new effective pulp capping agent. The benefits of successful pulp capping procedures would reduce the need for complex treatments, endodontic and restorative procedures. The basic knowledge from this study would encourage further research programs to develop and, finally, industrialize to a domestic commercial product.

1.2 Research question

Are there any effects of fluocinolone acetonide on type I collagen synthesis and *in vitro* calcification in human dental pulp cells?

1.3 Research objectives

1. To investigate the effect of fluocinolone acetonide on type I collagen synthesis in human cultured pulp cells.

2. To investigate the effect of fluocinolone acetonide on *in vitro* calcification in human cultured pulp cells.

1.4 Hypothesis

1. Null hypothesis H_0: fluocinolone acetonide has no effect on cultured human dental pulp cells with respect to the level of type I collagen synthesis.

Alternative hypothesis H_1: fluocinolone acetonide affects the cultured human dental pulp cells with respect to type I collagen synthesis.

2. Null hypothesis H_0: fluocinolone acetonide has no effect on cultured human dental pulp cells with respect to *in vitro* calcification.

Alternative hypothesis H_1: Fluocinolone acetonide affects the cultured human dental pulp cells with respect to *in vitro* calcification.

1.5 Experimental design

- Colorimetric assay for cell proliferation

- Type I collagen synthesis: Western blot analysis and reverse transcription polymerase chain reaction

- *In vitro* calcification

1.6 Key words

Dental pulp, healing, collagen, calcification, fluocinolone acetonide

1.7 Research design

Laboratory experimental research

1.8 Limitations of research

- The experimental design is an *in vitro* study using human cultured pulp cells. Effects of the interventions in this experiment cannot be completely judged to the populations.

- The results from the experiment cannot be used to explain the whole mechanism of pulp healing from the interventions. The mechanisms of pulpal healing *in vivo* are more complex, involving both cellular and extracellular events. Therefore, further experiments have to be investigated.

1.9 Benefits

1. To reveal the effect of fluocinolone acetonide on type I collagen synthesis and *in vitro* calcification in human cultured pulp cells.

2. To evaluate the possibility of fluocinolone acetonide whether it can be used as a new pulp capping material.

3. To obtain basic knowledge for further studies in the development of a new pulp capping material or use of topical corticosteroids in another applications.

1.10 Ethical consideration

There was no ethical problem because human teeth were obtained from caries-free impacted molars which extracted for orthodontic reason with patient's informed consent at the department of oral surgery, Faculty of Dentistry, Chulalongkorn University.