CHAPTER 1

Introduction

1. Backgrounds and rationale

Treatment of immature teeth with necrotic pulp and periapical pathosis presents several challenges. Many techniques, including calcium hydroxide ($Ca(OH)_2$) apexification and mineral trioxide aggregate (MTA) apical barrier formation, have been performed to close the open apex of immature teeth (1-5). Nonetheless, the dentinal walls of immature teeth still remain thin, short and weak and make these teeth susceptible to root fracture (6). Currently, the pulp revascularization procedure has been introduced as a new approach to manage immature teeth with necrotic pulp. The principles of this procedure consist of root canal disinfection, tissue generation, and the prevention of reinfection by creating a bacteria-tight seal (7-10). Continuation of root development and hard tissue deposition has been reported using this protocol (8-10). However, some studies have reported negative findings (11, 12). Many studies have revealed that tissue generated inside root canals following the revascularization procedure is not dental pulp (12-14).

One hypothesis for the continuation of root development following the revascularization procedure is that the remaining apical papilla tissue contains mesenchymal stem cells, which are the source of odontoblasts for root dentin formation (15, 16). Several studies have reported the capacity of stem cells of the apical papilla (SCAPs) to generate dentin-like tissue on the root canal walls (16, 17). Therefore, the attachment of SCAPs and their differentiation on the root dentin may play an important role in root continuation, including tissue generation in the revascularization procedure. So, the effects of medication and techniques used in all regenerative procedures should be as little as possible on the attachment of SCAPs to the root dentin. However, recent studies have reported the cytotoxicity of medications used in the revascularization technique (18-20). 3Mix, a combination of Metronidazole, Ciprofloxacin and

Minocycline, is widely used in revascularization (9, 10, 21, 22). Cheunsombat and colleagues (18) reported that 3Mix at high concentrations caused dental pulp and apical papilla cell death, but that 0.39 µg/mL of 3Mix is the best dose for cell proliferation without any toxicity. Ruparel and colleagues (20) also found that 3Mix at high concentrations had a detrimental effect on the survival of SCAPs, whereas lower concentrations (0.1 and 0.01 mg/mL) had no effect on cell viability. In that study, they reported that $Ca(OH)_2$ appeared to promote the proliferation of SCAPs at all concentration, particularly at 1 mg/mL.

Several studies also examined the effects of endodontic irrigants used in revascularization on cytotoxicity and cell attachment to root canal dentin (23-26). In 2011, Trevino *et al* (26) investigated the effects of various irrigation protocols on SCAP survival and attachment to the root canal wall. They revealed that treating the root canal surface with 6% sodium hypochlorite (NaOCl) or 2% chlorhexidine (CHX) had a detrimental effect on SCAPs, whereas 17% ethylene-diamine-tetra-acetic acid (EDTA) appeared to promote the SCAPs' viability and attachment to the root canal dentinal wall. Another similar study by Pang *et al* (24) in 2014 reported that treatment of the dentin surface with EDTA induced dental pulp stem cells' (DPSCs) attachment and odontoblastic/osteoblastic differentiation. The expression level of fibronectin 1, the important marker for cell attachment to root dentin, was significantly higher in EDTA-treated dentin than in untreated dentin. They also concluded that EDTA is beneficial for achieving successful outcomes in regenerative endodontics.

However, the effect of 3Mix as well as $Ca(OH)_2$ on cell attachment to root dentin has not been investigated. Therefore, in this study, the effects of 3Mix and $Ca(OH)_2$ (at various concentrations) on cell attachment to root canal dentin were examined. This study aimed to evaluate the ability of apical papilla cells (APCs) to attach to dentin surfaces that are treated with 3Mix or $Ca(OH)_2$ using fibronectin immunocytochemistry staining, alamarBlue® viability assay and morphological evaluation using scanning electron microscopy (SEM).

2. Research questions

- Is the attachment ability of APCs on human root dentin surfaces affected by treating dentin surfaces, either with 3Mix or $Ca(OH)_2$?

- Is the viability of APCs on human root dentin surfaces affected by treating dentin surfaces, either with 3Mix or $Ca(OH)_2$?

- Is the morphology of APCs on human root dentin surfaces affected by treating dentin surfaces, either with 3Mix or $Ca(OH)_2$?

3. Objectives of the study

- To evaluate the attachment ability of APCs to human root dentin surfaces treated either with 3Mix or $Ca(OH)_2$ in various recommended concentrations.

- To identify the expression of fibronectin of APCs on treated human root dentin surfaces.

- To evaluate the viability of APCs on treated human root dentin surfaces.

- To observe cellular attachment and morphology of APCs on treated human root dentin surfaces.

4. Research hypotheses

- The attachment ability of APCs to human root dentin surfaces is affected by treating dentin surfaces, either with 3Mix or $Ca(OH)_2$.

- The viability of APCs on human root dentin surfaces is affected by treating dentin surfaces, either with 3Mix or $Ca(OH)_2$.

- The morphology of APCs on human root dentin surfaces is affected by treating dentin surfaces, either with 3Mix or $Ca(OH)_2$.

5. Expected outcomes

- The results of this study should demonstrate the effects of 3Mix and $Ca(OH)_2$ on APC attachment to treated human root dentin.

- The results of this study should be the guidance to develop a treatment protocol for regenerative procedures.

6. Fields of research

This *in-vitro,* molecular biology study was performed in cell culture. The study was divided into three parts: Part I, fibronectin immunocytochemistry; Part II, alamarBlue® cell viability assay; and Part III, SEM observation. Human root dentin slices were divided in to six groups according to the medications added: PBS (no medication), 3Mix 0.39 µg/mL, 3Mix 100 µg/mL, 3Mix paste, $Ca(OH)_2$ 1 mg/mL, and $Ca(OH)_2$ 1,000 mg/mL, and treated for seven days. Thereafter, primary human APCs were seeded on the treated dentin slices. In Part I, APCs were cultured for four days and further investigated for their attachment ability on treated dentin, using fibronectin immunocytochemistry. The number of fibronectin-positive cells per microscopic field was counted. In part II, cell vitality assay was performed, using 15 µL of alamarBlue® added to the culture medium and the absorbance of the solution was monitored at specific time intervals: 0, 1, 2, 4, 6, 24, 48, and 72 hours. Part III was performed in order to observe the morphology of the APCs on the treated surfaces.