CHAPTER 1

INTRODUCTION

1. Backgrounds and rationale

The treatment of immature teeth with necrotic pulp is a challenge in dentistry. For many decades, clinicians have relied on conventional calcium hydroxide apexification or the use of artificial apical barriers to treat these teeth. Even though successful results have been reported (1-4), these procedures provided a small increase in root length and width after a period of time (5, 6). Therefore, these teeth are still susceptible to root fracture (7, 8). Recently, a revascularization method has been proposed as a new treatment protocol for immature teeth with necrotic pulps (9) since successful continuation of the tooth root has been radiographically reported (10-15).

Regenerative endodontics was first attempted, but unsuccessful, in the case series reported by Nygaard-Østby in 1961 concerning the role of blood clots in the wound healing process (16). In early 2000, a case report showing some interesting results in regeneration was published (10). In that case, an immature tooth diagnosed with necrotic pulp was treated and medicated using two antibiotics (metronidazole and ciprofloxacin). After 30 months, radiographic examination revealed dentin bridge formation, completion of the root apex, an increase in the thickness of the root canal wall and a positive response to electric pulp testing. Later, in 2004, Banchs and Trope proposed a new treatment protocol for the management of the immature tooth with

necrotic pulp, so called “revascularization.” (9). Briefly, a root canal was carefully disinfected, without mechanical disruption, using sodium hypochlorite (NaOCl), chlorhexidine (CHX), and a three-antibiotic combination (ciprofloxacin, metronidazole, and minocycline)(3Mix). After the signs and symptoms subsided (14 days), an intra-canal blood clot was stimulated through the root apex and then mineral trioxide aggregated (MTA) was placed over the blood clot. After using this technique, several case reports were published showing continuation of development of the tooth root together with increases in the root thickness (11-15). Since then, the clinical management protocol for infected immature permanent teeth has shifted.

One of the possible reasons why the development of root dentin can continue is that vital pulp cells may remain at the apical end of the root canal (9). These cells may proliferate into the created matrix and differentiate into odontoblasts. Another possible reason is the presence of mesenchymal stem cells (MSCs) in the apical papilla of permanent immature teeth. These stem cells are the source of odontoblasts, which are for the formation of root dentin (12). However, the exact pathway by which immature teeth continue root development has not yet been explored.

Recently, several studies describing the histological characteristics of teeth treated with the revascularization technique have been published (17-22). One interesting study in infected immature dog teeth treated with the revascularization procedure revealed that tissue grown into the root canal system after revascularization mainly resemble cementum, periodontal ligament (PDL) and bone. Only one case from 60 teeth in this study revealed partially survived pulp tissue (17).

A recent case report by Martin (2012) revealed the tissues regenerated in the canals were mineralized tissue resembling cementoid/osteoid and some fibrous connective tissue. No odontoblast-like cells, which are indicators of pulp-like tissue, were observed lining on the dentin wall (21).

Various reasons have been discussed regarding the ambiguous results in the current literatures (17, 23-25). One of those interesting reasons is that the high concentration of the three-antibiotic paste used might be toxic to living tissue (17). However, evidence of the toxicity of 3Mix in regenerative dentistry is still lacking, since most of the earlier studies focused on the antibacterial efficacy of 3Mix (26-28).

The clinical dosage of 3Mix generally used to date may be excessive and might affect the host tissue, causing cell death, which limits tissue regeneration. A recent study has shown that the three-antibiotic paste affected the viability of stem cells from the apical papilla (SCAPs) in a concentration-dependent manner. One milligram per millilitre of 3Mix caused 50% cell death (LC50), whereas the lower concentrations of 0.1 and 0.01 mg/mL had no detectable effect on SCAP viability (29). Furthermore, another study by Cheunsombat et al (2013) reported that cytotoxicity of 3Mix was induced when concentration and time increased. The concentration of 3Mix at 0.39 µg/mL was the best candidate for use since it produced less cytotoxicity to human dental pulp cells (DPCs) and apical papilla cells (APCs) than higher concentrations, while it was able to significantly reduce bacteria isolated from necrotic teeth (30).

However, there is no evidence showing that DPCs or APCs, after treatment with this non-cytotoxic dose of 3Mix, would promote mineralization of dentin, a

property which is important for the regeneration process. Therefore, the aims of this study are: (1) to determine the proliferative capacity of DPCs/APCs after treatment with 0.39 µg/mL of 3mix for seven days; (2) to compare mineralization potentials of 3Mix-treated DPCs/APCs to untreated DPCs/APCs; and (3) to identify the mRNA expression of mineralization markers of 3Mix-treated and untreated DPCs/APCs.

2. Research question

- Would proliferative capacity and differentiation potential of human DPCs be affected after 7 days exposure of cells to 0.39 µg/mL of 3Mix?
- Would proliferative capacity and differentiation potential of human APCs be affected after 7 days exposure of cells to 0.39 µg/mL of 3Mix?
- What is the difference in mineralization markers between 3Mix-treated cells and untreated cells?

3. Objective of the study

- To determine the proliferative capacity of human DPCs/APCs after treatment with 0.39 µg/mL of 3mix for 7 days.
- To compare mineralization potential of 3Mix-treated DPCs/APCs and untreated DPCs/APCs.
- To identify the mRNA expression of mineralization markers of 3Mix-treated and untreated DPCs/APCs.

4. Research hypothesis

- 3Mix antibiotics at 0.39 µg/mL would not affect the proliferative capacity of human DPCs/APCs.

- 3Mix antibiotics at 0.39 µg/mL would not affect the mineralization potential of human DPCs/APCs.
- The dentino-/osteogenic gene expression of 0.39 µg/mL 3Mix-treated human DPCs/APCs would not be significant difference compared to their control groups**.**

5. Expected outcomes

- This study should reveal that the previously reported non-cytotoxic dose of 3Mix has effects or no effect on DPC/APC functions.
- The result of this study should clarify that the recommended concentration of 3Mix can be used safely without destroying regenerative capacity of the cells. Therefore, the pulp revascularization can be more predictably achieved.
- This study should improve the treatment regimen for pulp revascularization.

6. Fields of Research

This study is an *in-vitro* study which performed in cell cultures and molecular biology. The study was divided into three parts: Part I, the proliferation assay; Part II, the dentino-/osteogenic capacity; and Part III, a quantitative RT-PCR. For experimental group in all parts, DPCs/APCs were treated with 0.39 µg/mL for seven days. After the treatment period, regular medium was used to culture both types of cells in PartI. These cells were further investigated for their proliferative capacity compared to control groups using a colorimetric qualification of 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide (MTT) at days 1, 3, 5 and 7. In Part II, both DPCs and APCs in control groups and experimental groups were cultured in dentino-/oseteogenic differentiating media for 7, 14 and 21 days and

further analyzed for their mineralization potential using spectrophotometrically quantitative Alizarin-red S staining. Part III was designed to evaluate mRNA expression of mineralization markers of 3Mix-treated DPCs/APCs compared to untreated DPCs/APCs using quantitative RT-PCR.