## UNIVERSITI SAINS MALAYSIA GERAN PENYELIDIKAN UNIVERSITI PENYELIDIKAN LAPORAN AKHIR

# THE MECHANISM OF NFKB SIGNALLING IN ODONTOGENIC STEM CELL DIFFERENTIATION INDUCED BY BMP2 FOR DENTAL PULP TISSUE ENGINEERING

PENYELIDIK

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## MECHANISM OF NF-KB SIGNALING IN BMP-2 – INDUCED DENTAL STEM CELL ODONTOGENIC DIFFERENTIATION ON HUMAN AMNIOTIC MEMBRANE SCAFFOLD

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#### ABSTRACT

NF- $\kappa$ B signalling pathway is involved in the tooth organogenesis and eruption process. With the elimination of NF- $\kappa$ B pathway could lead to a developmental detention of teeth. Hence, this study was conducted to investigate the mechanism of NF- $\kappa$ B signalling in the differentiation of SHED into odontoblast-like cells. Analysis of NF-  $\kappa$ B signalling was divided into two phases. The first phase was aimed to identify the optimal concentration of inhibitors. The second phase of the study was aimed to investigate the expression of stem cell, odontogenic, and NF- $\kappa$ B gene markers. Further analyses were carried out using real time RT-PCR. In conclusion, based on the gene expression analysis, this study suggested that inhibition of NF- $\kappa$ B directly involves in odontogenic differentiation of SHED when cultured on HAM with the treatment of BMP-2.

#### 1. INTRODUCTION

Dental caries is the most prevalent infectious disease among children and adults. This oral problem affects the quality of life of approximately 90% of the world's population (López and Baelum, 2007). Dental caries is characterised by infected and necrotic dental pulp tissue (Cooper *et al.*, 2010). The dental pulp tissue provides vitality and sensitivity to the tooth. The pulp tissue is highly vascularised, innervated and also serves as a source of stem cells. These characteristics enable the pulp to play a significant role in homeostasis and formation of reparative dentin (Galler *et al.*, 2011).

These drawbacks in conventional therapy can be overcome by the use of tissue engineering (TE) strategies to regenerate the dental pulp. The three core factors that ensure the success of TE are the types of cells, scaffold and growth factors used (Toda *et al.*, 2007). Stem cell research has become a new era and is regarded as one of the important fields for the understanding of tissue regeneration and its implementation in regenerative medicine. Stem cells exist in an undifferentiated state, capable of proliferating over extended periods of time through self-renewing divisions and later differentiating into a variety of cells that contribute to organ formation and function (Chagastelles and Nardi, 2011). Since the discovery and characterisation of multipotent mesenchymal stem cells (MSCs) from bone marrow, it remains an exciting prospective cell source for regenerative medicine applications because of their strong proliferative potential and multi-lineage differentiation capability. Moreover, the identification of stem cells from several dental tissues has made pulp tissue regeneration a realistic clinical possibility.

Despite the many types of dental stem cells available such as dental pulp stem cells (DPSCs) (Gronthos *et al.*, 2000), periodontal ligament stem cells (PDLSCs) (Gould *et al.*, 1977; Gronthos *et al.*, 2006), stem cells from the apical papilla (SCAP) (Sonoyama *et al.*, 2006), and stem cells from human exfoliated deciduous teeth (SHED) (Miura *et al.*, 2003). This study was carried out on the SHED since the focus of this research is odontogenesis. Stem cells from dental pulp are categorised under adult stem cells and derived from ectoderm (Ulmer *et al.*, 2010). It exhibits the MSC properties which has a self-renewal ability and able to transdifferentiate into another type of cell (Huang *et al.*, 2009a).

### 2. RESEARCH METHODOLOGY

This is an *in vitro* experimental study. The first part of this study includes protein extraction from SHED treated with NF-kB inhibitors and determination of suitable concentration of inhibitors to inhibit the NF-kB pathway. In the second part, gene expression analyses of stem cells, odontoblasts and NF-kB gene markers were evaluated on SHED cultured with and