THE STUDY OF DENTAL PULP STEM CELLS FOLLOWING RESTORATIVE PROCEDURES ON DECIDUOUS MOLARS

by

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DEDICATION

I dedicate this thesis to my parents. I hope that this achievement will help me to complete the dream that you have for me in all those many years ago when you chose to give me the best education you could.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

α-MEM	alpha Modified Eagle's Medium
AAPD	American Academy of Pediatric Dentistry
AB	AlamarBlue
BMP	Bone Morphogenic Protein
BMSCs	Bone Marrow Stem Cells
BrdU	Bromodeoxyuridine
$Ca(OH)_2$	Calcium hydroxide
CD	Cluster of Differentiation
CFU-F	Colony Forming Unit-Fibroblast
CO_2	Carbon dioxide
DAB	Diaminobenzidine
DPBS	Dulbecco's Phosphate Buffered Saline
DPSCs	Dental Pulp Stem Cells
EDTA	Ethylenediamine Tetra Acetic Acid
ELIZA	Enzyme Linked Immunosorbent Assay
ESCs	Embryonic Stem Cells
FBS	Fetal Bovine Serum
FGF	Fibroblast Growth Factor
GIC	Glass Ionomer Cement
H&E	Haematoxyline and Eosin
HBSS	Hank's Balanced Salt Solution
HRP	Horseradish peroxidase
HSCs	Hematopoietic Stem Cells
Ig	Immunoglobulin
IGF	Insulin like Growth Factor
IPC	Indirect Pulp Capping
LPS	Lipopolysaccharide
MTA	Mineral Trioxide Aggregate
PBS	Phosphate Buffered Saline
PDLSCs	Periodontal Ligament Stem Cells
RMGICs	Resin Modified Glass Ionomer Cements
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SHED	Stem cells from Human Extracted Deciduous teeth
TDT	Tertiary Dentin Thickness
TGF-ß	Transforming Growth Factor-ß
USM	Universiti Sains Malaysia

KAJIAN KE ATAS SEL STEM PULPA GIGI SELEPAS PROSEDUR RAWATAN RESTORATIF PADA MOLAR-MOLAR GIGI DESIDUS

ABSTRAK

Pemeriksaan biologi untuk kecederaan pulpa, memperbaiki kerosakan dan respon sel-sel stem pulpa gigi terhadap bahan-bahan yang digunakan untuk pemulihan gigi amat penting bukan hanya ketika memahami perkembangan gigi selepas pembedahan tetapi juga ketika menjalankan aktiviti-aktiviti pembaikan untuk menyempurnakan rawatan pemulihan. Objektif kajian ini adalah untuk membandingkan aktiviti pembiakan sel stem dengan menggunakan kolometrik reagen pembiakan alamarBlue® dan untuk menilai respon sel-sel tersebut dengan membandingkan pembentukan Ketebalan Dentin Tersier (TDT) dengan menggunakan pewarna 'Haematoxyline' dan 'Eosin' untuk pemeriksaan histologi, selepas prosedur pemulihan molar gigi desidus dilakukan. Mereka yang di pilih terdiri daripada pesakit pergigian pediatrik yang sihat yang datang ke klinik pergigian pediatrik untuk rawatan gigi di Pusat Pengajian Sains Pergigian, Kampus Kesihatan, Universiti Sains Malaysia, berumur 9 hingga 11 tahun, mempunyai karies molar-molar gigi desidus tetapi pulpanya tidak terdedah. Gigi-gigi tersebut telah dibahagikan kepada dua kumpulan; Kumpulan 1 terdiri daripada 29 batang gigi yang ditampal dengan simen Ionomer Kaca (GIC) sahaja dan Kumpulan 2 pula terdiri daripada 29 batang gigi yang dilapik dengan simen Kalsium Hidroksida ($Ca(OH)_2$) dan kemudiannya ditampal dengan GIC. Gigi-gigi tersebut telah dikaji selama 6 bulan sebelum dicabut mengikut prosedur piawaian berdasarkan kriteria yang telah

ditentukan. Sejumlah 34 batang gigi telah dicabut, 17 batang gigi untuk setiap kumpulan. Pada setiap kumpulan yang dikaji, 7 batang gigi telah dihantar untuk kultur sel stem, manakala 10 batang gigi lagi menjalani keratan histologi. Keputusan menunjukkan bahawa sel-sel stem daripada gigi susu manusia yang telah dicabut (SHED) adalah positif untuk petunjuk permukaan antigenik, CD105 dan CD 106, selepas ujian imunositokimia dilakukan. Aktiviti pembiakan (Median, IQR) untuk Kumpulan 1 (38, 19) adalah kurang jika dibandingkan dengan Kumpulan 2 (41,19) dan ia adalah tidak signifikan ($p>0.05$). Walau bagaimanapun, kawasan TDT (Median, IQR) untuk Kumpulan 2 (0.15, 0.033) adalah lebih tinggi jika dibandingkan dengan Kumpulan 1 (0.05, 0,013) dan ia adalah signifikan ($p<0.001$). Pulpa gigi desidus mengandungi populasi sel stem, dan sel-sel tersebut menunjukkan respon yang baik serta dapat mengekalkan aktiviti pembiakan mereka selepas prosedur pemulihan menggunakan simen $Ca(OH)_2$ dan GIC. Walau bagaimanapun, bahan-bahan ini mewujudkan respon yang berbeza pada pemendapan dentin tersier dan pemendapan dentin reaksioner kelihatan lebih pada pemulihan gigi yang dilapik simen $Ca(OH)_2$. Oleh sebab itu, simen $Ca(OH)_2$ mempunyai kredibiliti yang lebih baik untuk digunakan ketika merawat pesakit yang mempunyai kaviti yang dalam, untuk perlindungan pulpa.

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ABSTRACT

The biological examination of pulp injury, repair events and the response of dental pulp stem cells to dental restorative materials is of paramount importance for understanding the post operative development and the use of these repair activities to accomplish restorative treatment. The objectives of this study were to compare the stem cells proliferative activity using colorimetric proliferation reagent alamarblue® and to evaluate their response by comparing the Tertiary Dentin Thickness (TDT) formation using Haematoxyline and Eosin staining for histological examination following restorative procedures on deciduous molars. Healthy paediatric dental patients aged between 9 to 11 years with caries deciduous molars without pulpal exposure, who attended the paediatric dental clinic for dental treatment in the School of Dental Sciences, Health Campus, Universiti Sains Malaysia, were selected. The teeth were divided into two groups; *Group 1* comprised of 29 teeth filled with Glass Ionomer Cement (GIC) alone and *Group 2* with 29 teeth, lined with Calcium hydroxide ($Ca(OH)_2$) cement and filled with GIC. The teeth were reviewed for up to 6 months before being extracted according to standardized procedures based on the selected criteria. A total of 34 teeth were extracted with 17 teeth belonging to each group. In each study group 7 teeth were assigned for stem cell culture and 10 teeth were subjected to histological sections.

The results showed that stem cells from human extracted deciduous teeth (SHED) were positive for surface antigenic markers, CD105 and CD166 following

immunocytochemistry test. The proliferative activity (Median, IQR) for *Group 1* (38,19) was less as compared to *Group 2* (41,19) and it was not significant ($p>0.05$). However, TDT area (Median, IQR) in *Group 2* (0.15,0.033) was higher as compared to *Group 1* (0.05,0.013) and it was significant ($p<0.001$). The pulp of deciduous teeth contains stem cell population, which respond well and maintains proliferative activity following restorative procedures using $Ca(OH)_2$ cement and GIC. However, these materials create different response in tertiary dentin deposition, where the reactionary dentin deposition appeared to be more under restorations lined with $Ca(OH)_2$. Thus $Ca(OH)_2$ cement has high credibility to be used in deep cavities for pulp protection.

CHAPTER ONE

INTRODUCTION

1.1 Background of study

The management of the child dental patient is geared towards the preservation of both the primary and permanent dentition. The primary dentition is important for stimulating the development of the dental arches, maintaining normal occlusion relationship and speech development (Welbury and Walton, 1999). The requirements for a restorative material in the primary dentition are different from those in the permanent dentition and a material which is ideal for one may not be ideal for the other. Primary teeth have a shorter life span of only about 8-9 years and hence, restorations placed in the teeth remains in the oral environment only for a shorter period of time.

In 2005, American Academy of Pediatric Dentistry (AAPD) recommended that any restorative material being used should seal completely the involved dentin from the oral environment and preserve dental pulp vitality. The complete seal can reduce post-treatment signs or symptoms such as sensitivity, pain or swelling. The post-treatment effects can be assessed using radiograph, where there should be no evidence of pathological external or internal root resorption or other pathological changes.

It is well documented that the use of adhesive materials like glass ionomer cement (GIC), resin modified GICs and polyacid modified composite resins in children allows less destructive cavity preparation and smaller restoration. This subsequently reduces treatment time and may limit the use of local analgesia. The advantages of using these adhesive restorative materials are liberation of long term fluoride release and the ability to be recharged by exposure to fluoride solutions and gels (Bilgin and Ozalp, 1998).

Furthermore, teeth restored with GIC showed less recurrent caries formation (Mount, 1995).

Teeth with deep cavities needs a protective base material such as calcium hydroxide ($Ca(OH)_2$) to be placed on the pulpal surface of a cavity preparation in order to cover exposed dentin tubules and act as a protective barrier between the restorative material or lining cement and the pulp of the tooth. $Ca(OH)_2$ has the ability to stimulate tertiary dentin in indirect pulp contact and dentinal bridge formation with direct pulp contact and serve as a protective barrier for pulp tissue by blocking patent dentinal tubules and by neutralizing the attack of inorganic acids and leached products from certain cements. These advantages lead to wide usage of this material in dental practice (Stanley and Pameijer, 1997).

In 2003, Miura and co-workers identified stem cells from pulp of human primary teeth, named as SHED (Stem Cells from Human Exfoliated Deciduous teeth) and these cells have been shown to divide continuously and differentiate into a variety of other cell types, including nerves, fat, and tooth-generating cells. In addition, deciduous teeth may provide an ideal source of stem cells which can proliferate and differentiate into dentin-forming odontoblasts (Nakashima, 1994; Gronthos *et al.*, 2002). Damaged odontoblasts can be replaced by newly generated populations of odontoblasts derived from pulp. Following physiological stimulation or injury, such as caries and operative procedures, stem cells in pulp may be mobilized to proliferate and differentiate into odontoblasts by morphogens released from the surrounding dentin matrix (Tziafas *et al.*, 2000). The technique in tissue engineering with the applications of dental pulp progenitor/stem cells, morphogens, and scaffolds triad may provide a useful alternative method for pulp-capping and root canal treatment (Nakashima and Reddi, 2003).

1.2 Statement of problem

The long-term goal of dental treatment is to preserve teeth and prolong their function. However, in the United States alone, despite the effectiveness of preventive dentistry and dental health care, 290 million fillings are placed each year and close to 200 million restorations or two-thirds of these restorations have failed (Murray *et al.*, 2002f). Moreover, significant percentages of these restored teeth ultimately undergo pulpal necrosis, requiring either tooth extraction or endodontic treatment and prosthodontic crown buildup (Edwards and Mason, 2006).

Therefore, it is necessary to evaluate the changes which take place in teeth, such as responses of dental pulp following restorative procedures as well as its reactions to restorative materials. However, to our knowledge, there is limited data currently available regarding the biological response of stem cells to various dental restorative materials such as GIC and lining materials such as $Ca(OH)_2$.

1.3 Justification of the study

The biological examination of pulp injury, repair events and response of dental pulp stem cells to restorative materials is of paramount importance for understanding the post operative success and problems and knowledge of these responses can be used to improve restorative treatment modalities. The tooth structure is exposed to different dental materials in different restorative procedures. Hence, this study aims to evaluate the response of dental pulp stem cells to some of the most commonly used dental materials in pediatric dentistry, such as GIC and $Ca(OH)_2$.

1.4 Objectives of the study

1.4.1 General objective

To study the dental pulp stem cells following restorative procedures on deciduous molars.

1.4.2 Specific objectives

1. To compare the dental pulp stem cells' proliferative activity between teeth filled with GIC alone and teeth lined with $Ca(OH)_2$ cement and filled with GIC by using colorimetric cell proliferation reagent alamarBlue®.
2. To compare the thickness of tertiary dentin area that form between teeth filled with GIC alone and teeth lined with $Ca(OH)_2$ cement and filled with GIC by histological staining.

1.4.3 Working hypothesis

1. The stem cells from human extracted deciduous teeth showed high proliferative activity following restorative procedure using $Ca(OH)_2$ cement as a liner and filled with GIC as compared to the restorative procedure using GIC alone.
2. The tertiary dentine thickness area following restorative procedure using $Ca(OH)_2$ cement as liner and filled with GIC is thicker as compared to the restorative procedure using GIC alone.

CHAPTER TWO

LITERATURE REVIEW

2.1 Restorative treatment for primary teeth

The primary objective of restorative treatment is to restore the teeth to a healthy, functional and esthetic state as well as to prevent the recurrence of caries. The indications, objectives and type of pulpal therapy depend on whether the pulp is vital or nonvital (McDonald *et al.*, 2004). Pulp vitality can be clinically diagnosed by dental history, visual evaluation, radiograph and additional tests such as palpation, percussion and mobility evaluation. Therefore, depending on the diagnosis and tests, the restorative treatment for primary teeth can be either vital pulp therapy or non vital pulp therapy.

2.1.1 Vital pulp therapy for primary teeth

The aim of vital pulp therapy is to maintain the vitality of the pulp. Teeth exhibiting provoked pain of short duration which is relieved upon the removal of the stimulus or with analgesics should be treated by vital pulp therapy. This can be done by many methods such as indirect pulp capping treatment which is performed in deep carious lesions adjacent to the pulp. In the indirect pulp capping procedure the carious dentin near the pulp is left in place to avoid pulp tissue exposure and covered with radiopaque biocompatible base materials such as $Ca(OH)_2$, zinc oxide eugenol and GIC, to stimulate healing and repair (Farooq *et al.*, 2000). However, when there is a small traumatic exposure of the pulp during cavity preparation of vital non-infected pulp, direct pulp capping procedure was performed by controlling the pulp bleeding and capping or covering the exposed pulp with $Ca(OH)_2$ cement or mineral trioxide aggregate (MTA). It has been suggested that the high cellular content of primary pulp

tissue may be responsible for the failure of direct pulp capping in primary teeth. Therefore, this treatment is considered as a contra-indication for the primary teeth due to post operative complications such as pulpal inflammation, pulp necrosis or internal resorption (Carrotte, 2005).

Pulpotomy is used as an alternative procedure to overcome the failure of direct pulp capping procedure in primary teeth and defined as extirpation of vital inflamed pulp from the coronal chamber followed by medicament placement over radicular pulp stumps. Pulpotomy is indicated when caries removal results in pulp exposure in a primary tooth with a normal pulp or reversible pulpitis or after a traumatic pulp exposure. The materials that were used in this method are fomocresol, glutaraldehyde, $Ca(OH)_2$ and ferric sulfate (Nicky *et al.*, 1997).

2.1.2 Nonvital pulp therapy for primary teeth

Nonvital pulp therapy is carried out to maintain the teeth in the oral cavity as natural space maintainer, which can reduce the complications of premature loss of teeth such as space loss, eruptive difficulties, mesial migration of permanent premolars, canines blocked-out and microdontia (Northway, 2000). Teeth exhibiting signs and/or symptoms such as a history of spontaneous unprovoked tooth ache, a sinus tract, periodontal inflammation not resulting from gingivitis or periodontitis, excessive mobility not associated with trauma or exfoliation, furcation/apical radiolucency, or radiographic evidence of internal/external resorption. The above types of teeth tend to be treated by nonvital pulp therapy. This can be obtained by method called pulpectomy, which can be defined as root canal procedure when the pulp is either non-vital or irreversibly inflamed.

Pulpectomy is indicated in a primary tooth with carious pulp exposures and hyperaemia or persistent bleeding during pulpotomy procedure. In this case, radicular pulp should be removed and the root canals are debrided, enlarged, disinfected and filled with materials such as non-reinforced zinc oxide eugenol. Pulpectomy is considered difficult because of the complexity of the root canals in primary molars and over instrumentation may lead to injury of the subjacent permanent tooth germs (Barr *et al.*, 1991).

2.2 Secondary dentin reaction to restorative procedure

There are many reactions and changes in remaining secondary dentin after cavity preparation procedure and restorative material placement. These reactions and changes in remaining secondary dentin should be taken into consideration because they can affect the restorative procedure as well as the restorative material that has been placed. The physical process of cavity preparation with hard instruments or burr to the tooth remaining secondary dentin results in the formation of the smear layer (less than 2μm in thickness). Smear layer reduces the fluid flow from dentin, which may have a protective effect on the pulp tissue. But this layer is not a stable structure and since bacteria can be found in it, complete removal of this layer may be necessary to obtain optimal chemical and mechanical bonding between restorative materials and tooth structures. Since smear layer cannot be completely removed by a water spray or by scrubbing, other materials and techniques are available to assure the complete removal of smear layer such as acid-etching procedures, application of pumice to the prepared remaining secondary dentin surface and by ethylenediamine tetra acetic acid (EDTA) (Sim and Sidhu, 1994).

It has also been established that preparation techniques and cutting secondary dentin by bur lead to generation of heat and this heat-energy is the most injurious event to pulp tissue. Moreover, few investigations have found that the amount of intra-pulpal injury generated during cavity preparation depends on the drill rotation speed, size, type and shape of cutting instrument, length of time the instrument is in contact with the secondary dentin, amount of pressure and cooling techniques (Murray *et al.*, 2002e).

Separation of the secondary dentin and pulp and displacement of odontoblastic nuclei into dentinal tubules can occur when inadequate cooling procedure during cavity preparation is used. Excessively dried remaining secondary dentin may show disorganization in the organelles of the odontoblasts and in the adjacent cells. Over heating or burning of remaining secondary dentin during the cavity preparation lead to color change in the margin of the preparation and is considered as the most common reason for displacement of cells into dentinal tubules and causing disruption of the contents of the tubules (Mjor and Odont, 2001). The disturbance and disorganization event of the cells as mentioned above is considered as gross reaction to injury and the result of these events is the degeneration of the odontoblast processes which are likely to have an inflammatory effect on the adjacent pulpal tissue (Smith *et al.*, 1995).

Vascular responses have been demonstrated by peripheral flow of dentinal fluid following cavity preparation, which allows plasma protein to enter the tubules. Clotting of these proteins will reduce the functional diameter of the remaining secondary dentin tubules and reduce the permeability of the dentin, which may be clinically important in preventing bacteria and toxic agents from diffusing into the pulp (Pashley *et al.*, 1983).

The ability of the secondary dentin to respond to caries, attrition, abrasion, erosion, dental restorative procedures and materials by hard tissue formation has long been recognized. Such responses are often seen as focal deposition of the tertiary dentin matrix beneath the site of injury, which act as a barrier and protect the pulp injurious challenge by increasing the distance of the pulp from the noxious stimulus causing an overall decrease in remaining secondary dentin permeability (Murray *et al.*, 2002f).

The tertiary dentin matrix can be variable in quality in term of tubular structure, which clearly influence dentin permeability in this region. The barrier properties of focal tertiary dentin matrix will be advantageous in both protecting the pulp vitality and also, in providing a sounder hard-tissue base for subsequent restoration of the injured tissue (Tziafas *et al.*, 1998).

The nature of tertiary dentinogenic responses may be important in determining the properties of the new matrix. In case of mild injury to the tooth (injurious challenge not reach the pulp tissue), the odontoblasts responsible for primary dentin secretion can often survive the injurious stimulus and are stimulated to secrete a reactionary dentin matrix focally to the pulp- dentin interface beneath the injury site. Since the original primary odontoblasts are responsible for this matrix secretion, there will be tubular continuity and communication with the primary dentin matrix. With more severe injury, odontoblasts beneath the injury may die, and if suitable conditions prevail within the pulp, a new generation of odontoblasts-like cells may differentiate from the underlying pulpal cells secreting a reparative dentin matrix. Matrix secretion from new generation of cells implies discontinuity in tubular structure with subsequent reduction in dentin permeability (Murray *et al.*, 2002f).