UNIVERSITI SAINS MALAYSIA
GERAN PENYELIDIKAN UNIVERSITI PENYELIDIKAN
LAPORAN AKHIR

ROLE OF TRANSFORMING GROWTH FACTOR-BETA1 (TGFB-1) AND THE CELL SIGNALLING PATHWAY IN THE DIFFERENTIATION OF SHED INTO EPITHELIAL-LIKE CELLS

PENYELIDIK

DR. AZLINA AHMAD

PENYELIDIK BERSAMA

ASSOC. PROF. DR. TP KANNAN
ASSOC. PROF. DR. KHAIRANI IDAH MOKHTAR @ MAKHTAR
DR. ZURAIRAH BERAHIM
NUR IZYAN AZMI

2017

KEMENTERIAN PENDIDIKAN MALAYSIA

FINAL REPORT
FUNDAMENTAL RESEARCH GRANT SCHEME (FRGS)
Laporan Akhir Skim Geran Penyelidikan Fundamental (FRGS)
Pindaan 1/2015

ESEARCH TITLE: **Role of Transforming Growth Factor Beta1 (TGFβ-1) and the cell signaling pathway in the differentiation of SHED into epithelial-like cells.**

HASE & YEAR: **3 & 2015**

TART DATE: **1 June 2012**
ND DATE: **31 Mei 2014**
XTENSION PERIOD (DATE): **RMC LEVEL: 30 November 2014**
KPM LEVEL: 31 May 2015

ROJECT LEADER: **Dr Azlina Ahmad**
C / PASSPORT NUMBER:

ROJECT MEMBERS: (including GRA)
1. **AP Dr TP Kannan**
2. **AP Dr Khairani Idah Mokhtar @ Makhtar**
3. **Dr Zurairah Berahim**
4. **Nur Izyan Azmi**

JECT ACHIEVEMENT *(Prestasi Projek)*

ACHIEVEMENT PERCENTAGE			
Project progress according to milestones achieved up to this period	0 - 50%	51 - 75%	76 - 100%
Percentage (please state #%)			90%

RESEARCH OUTPUT		
Number of articles/ manuscripts/ books *(Please attach the First Page of Publication)*	Indexed Journal	*Non-Indexed Journal*
		1
Conference Proceeding *(Please attach the First Page of Publication)*	International	*National*
	1	2
Intellectual Property *(Please specify)*		

HUMAN CAPITAL DEVELOPMENT

Human Capital	Number				Others (please specify)
	On-going		Graduated		
Citizen	Malaysian	Non Malaysian	Malaysian	Non Malaysian	
No. PHD STUDENT					
Student Fullname: IC / Passport No: Student ID:					
No. MASTER STUDENT	1				
Student Fullname: IC / Passport No: Student ID:					
No. UNDERGRADUATE STUDENT					
Student Fullname: IC / Passport No: Student ID:					
Total	1				

EXPENDITURE *(Perbelanjaan) as Borang K1(RMC)*

C

Budget Approved *(Peruntukan diluluskan)* :
Amount Spent *(Jumlah Perbelanjaan)* :

Balance *(Baki)* :
Percentage of Amount Spent :
(Peratusan Belanja)

ADDITIONAL RESEARCH ACTIVITIES THAT CONTRIBUTE TOWARDS DEVELOPING SOFT AND HARD SKILLS
(Aktiviti Penyelidikan Sampingan yang menyumbang kepada pembangunan kemahiran insaniah)

D

International

Activity	Date (Month, Year)	Organizer
(e.g : Course/ Seminar/ Symposium/ Conference/ Workshop/ Site Visit)	1. International Conference On Medical & Health Sciences (ICMHS) 22-24 May 2013	1. College of Pathologists, Health Campus, USM.

National

Activity	Date (Month, Year)	Organizer
(e.g : Course/ Seminar/ Symposium/ Conference/ Workshop/ Site Visit)	1. Health and Life Sciences Postgraduate Conference 2014 10-11 June 2014 2. 10th Malaysia Genetics Congress 2013 (MGC10) 3-5 December 2013	1. Institute for Research in Molecular Medicine (INFORMM) 2. Persatuan Genetik Malaysia

E **PROBLEMS / CONSTRAINTS IF ANY** *(Masalah/ Kekangan sekiranya ada)*

1. Protein expression analysis takes longer times than expected.

ECOMMENDATION (*Cadangan Penambahbaikan*)

ıture direction: Since this study showed that KGM media was not successfully to completely differentiated SHED into ɔithelial-like cells, we suggest that maybe addition of keratinocyte growth factor protein in the KGM media will help in e differentiation. However, we could not do this experiment because the growth factor is expensive.

ESEARCH ABSTRACT – Not More Than 200 Words (*Abstrak Penyelidikan – Tidak Melebihi 200 patah perkataan*)

ɔithelial cells plays crucial role in human body and in oral cavity, epithelial formation is important during the healing rocess after injuries. Previous study had shown that primary cells from human exfoliated deciduous teeth (SHED) can e differentiated into epithelial-like cells when cultured in Keratinocyte Growth Medium (KGM). This study aim to etermine the effects of TGF-β1 and ALK-5 inhibitor in the differentiation of SHED into epithelial-like cells cultured in ɔecific differentiation medium (KGM). MTT and alamarBlue assay was done to investigate and determine the best ɔncentration to be used in the study. Protein expression using flowcytometer was conducted to identify the presence of tem cell markers, CD105, and epithelial marker, E-Cadherin. The cell was positive for stem cell protein marker, but we ill optimizing the protein expression level of E-Cadherin. Then, gene expression of mesenchymal, epithelial, and TGF- signaling marker was carried out on SHED cultured with KGM treated with 1.25 ng/ml of TGF-β1 or 0.625 µM of ALK-5 hibitor. The treated SHED was harvested at day 1, 3, 7, 14, and 21 and subjected to RNA extraction. Then, study was receded with two-step Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). The presence of mesenchymal narker, *Nanog* on all samples except on SHED cultured in KGM + 0.625 µM on day 21. Based on epithelial marker, *eratin5* showed there is no expression on all treated samples. For TGF-β signaling marker which were *ALK-5* and *mad4*, *Smad4* showed expression on day 1 until 7 for all treatments and only SHED in KGM on day 14 showed a mild xpression. *ALK5* expressed in most of samples analysed but only SHED in KGM with TGF-β1 showed no expression. s conclusion, this study showed that TGF-β1 and ALK-5 inhibitor might give a little effect to the differentiation of SHED nto epithelial-like cells but not enough to induce the differentiation process.

)ate :
arikh

Project Leader's Signature:
Tandatangan Ketua Projek

H	**COMMENTS, IF ANY/ ENDORSEMENT BY RESEARCH MANAGEMENT CENTER (RMC)** *(Komen, sekiranya ada/ Pengesahan oleh Pusat Pengurusan Penyelidikan)*

.....

Pembentangan.

.....

.....

Name:
Nama:

PROF. DR LEE KEAT TEONG
Pengarah
Pejabat Pengurusan & Kreativiti Penyelidikan
Universiti Sains Malaysia

Signature:
Tandatangan:

Date:
Tarikh:

Title: Role of Transforming Growth Factor Beta1 (TGFβ-1) and the cell signaling pathway in the differentiation of SHED into epithelial-like cells.

ABSRACT

Epithelial cells plays crucial role in human body and in oral cavity, epithelial formation is important during the healing process after injuries. Previous study had shown that primary cells from human exfoliated deciduous teeth (SHED) can be differentiated into epithelial-like cells when cultured in Keratinocyte Growth Medium (KGM). This study aim to determine the effects of TGF-β1 and ALK-5 inhibitor in the differentiation of SHED into epithelial-like cells cultured in specific differentiation medium (KGM). MTT and alamarBlue assay was done to investigate and determine the best concentration to be used in the study. Then, gene expression of mesenchymal, epithelial, and TGF-β signaling marker was carried out on SHED cultured with KGM treated with 1.25 ng/ml of TGF-β1 or 0.625 μM of ALK-5 inhibitor. The treated SHED was harvested at day 1,3,7,14, and 21 and subjected to RNA extraction. Then, study was proceeded with two-step Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). The presence of mesenchymal marker, *Nanog* on all samples except on SHED cultured in KGM + 0.625 μM on day 21. Based on epithelial marker, *Keratin5* showed there is no expression on all samples treatment except control which is keratinocyte cells. For TGF-β signaling marker which were *ALK-5* and *Smad4*, *Smad4* showed expression on day 1 until 7 for all treatmets and only SHED in KGM on day 14 showed a mild expression. *ALK5* expressed in most of samples analysed but only SHED in KGM with TGF-β1 showed no expression. As conclusion, this study showed that TGF-β1 and ALK-5 inhibitor might give a little effects to the differentiation of SHED into epithelial-like cells but not enough to induce the differentiation process.