UNIVERSITI SAINS MALAYSIA
GERAN PENYELIDIKAN UNIVERSITI PENYELIDIKAN
LAPORAN AKHIR

"ANGIOGENIC POTENTIAL OF DENTAL STEM CELLS SEEDED ON HUMAN AMNIOTIC MEMBRANE AS A SCOFFOLD FOR PULP TISSUE REGENERATION"

PENYELIDIK

DR. AZLINA AHMAD

PENYELIDIK BERSAMA

PROF. MADYA DR. KHAIRANI IDAH BT MOKHTAR@MAKHTAR
PROF. DR. SUZINA BT. SHEIKH ABDUL HAMID
PROF. MADYA DR. T.P. KANNAN

2017

RESEARCH UNIVERSITY (INDIVIDUAL) GRANT REPORT

ANGIOGENIC POTENTIAL OF DENTAL STEM CELLS SEEDED ON HUMAN AMNIOTIC MEMBRANE AS A SCAFFOLD FOR PULP TISSUE REGENERATION

Investigators:
Dr Azlina Ahmad
Assoc. Prof. Dr. Khairani Idah Binti Mokhtar @ Makhtar
Prof. Dr. Suzina Bt. Sheikh Abdul Hamid
Assoc. Prof. Dr. T. P. Kannan

2017

Project Code :
(for RCMO use only)

UNIVERSITI SAINS MALAYSIA

RU GRANT
FINAL REPORT FORM

Please email a softcopy of this report to rcmo@usm.my

A	**PROJECT DETAILS**
i	**Title of Research:** Angiogenic Potential Of Dental Stem Cells Seeded On Human Amniotic Membrane As A Scaffold For Pulp Tissue Regeneration
ii	**Account Number:**
iii	**Name of Research Leader:** Dr Azlina Ahmad
iv	**Name of Co-Researcher:** 1. Assoc. Prof. Dr. Khairani Idah Binti Mokhtar @ Makhtar 2. Prof. Dr. Suzina Bt. Sheikh Abdul Hamid 3. Assoc. Prof. Dr. T. P. Kannan
v	**Duration of this research:** a) **Start Date** : 15 / 12 / 2012 b) **Completion Date** : 14 / 12 / 2016 c) **Duration** : 4 years d) **Revised Date** (*if any*) : 14 / 12 / 2016
B	**ABSTRACT OF RESEARCH**
	(An abstract of between 100 and 200 words must be prepared in Bahasa Malaysia and in English. This abstract will be included in the Report of the Research and Innovation Section at a later date as a means of presenting the project findings of the researcher/s to the University and the community at large) Bahasa Malaysia Perubatan generatif untuk merawat luka adalah penting kepada manusia. Tujuan kajian ini dijalanan adalah untuk mengkaji potensi pembentukan vaskular baharu menggunakan model 3D terdiri daripada membran amnion manusia (HAM), stem sel daripada gigi susu (SHED) dan juga faktor pertumbuhan vaskular endotelium (VEGF). SHED yang tumbuh di permukaan stroma HAM mampu untuk proliferat, migrasi dan membeza. Analisis SEM menunjukkan SHED yang dikultur pada permukaan HAM dengan penambahan VEGF berubah bentuk daripada bentuk fibroblas kepada bentuk bulat. Selain itu, analisis H&E menunjukkan SHED mempunyai keupayaan unutk membentuk lapisan mono pada permukaan stroma HAM hingga ke hari 14. Namun begitu, pada hari 21, sel mula memasuki ke dalam lapisan stroma HAM. Hal ini terjadi berkemungkinan disebabkan pembezaan sel telah berlaku di mana SHED

telah berubah saiz menjadi lebih kecil, membolehkan sel tersebut memasuki ruang tisu bergentian. Analisis gen juga menunjukkan berlakunya proses pembezaan endotelium. Walaupun tiada pengekspresan gen VEGFR2 dan CD31 berlaku, analisis protein menunjukkan pengekspresan protein cox-2. Cox-2 terlibat dalam pembentukan tubul. Ini menujukkan SHED berupaya untuk membeza dan terlibat dalam proses pembentukan vaskular baharu dengan adanya komponen matrik ekstrasel pada HAM.

English
Regenerative medicine, particularly in wound healing, is necessary for the mankind. Our study aim to investigate the angiogenic potential of a 3D model made up of Human Amniotic Membrane (HAM) scaffold; Human Exfoliated Deciduous Teeth (SHED) and Vascular Endothelial Growth Factor (VEGF). SHED grew on the stromal side of HAM had the ability to proliferate, migrate and differentiate. The SEM analysis revealed that SHED cultured on HAM with the addition of VEGF change its fibroblast-like shape into rounded-like shape. Besides, H&E analysis also showed the ability of SHED cell to form monolayer structure on stromal surfaces until day 14. However, on day 21, cells started to infiltrate inside the HAM stromal layer. The infiltration could happen due to cell differentiation, where SHED might change its size, allowing cells to invade inside this fibrous structure. Gene analysis also revealed the potential of endothelial-like differentiation. Even though there was no expression of VEGFR2 and CD31 genes, protein analysis showed the expression of Cox-2. The Cox-2 expression is involved in the formation of the tubule. All the results proved that SHED has a potential to differentiate and involved in angiogenesis with the help of extracellular matrix components on HAM.

C BUDGET & EXPENDITURE

i

Total Approved Budget :

Total Expenditure :

Balance :

Percentage of Amount Spent (%) :

 # Please attach final account statement (eStatement) to indicate the project expenditure

ii Equipment Purchased Under Vot 35000

No.	Name of Equipment	Amount (RM)	Location	Status
1			Craniofacial Science Laboratory, PPSG, USM	Active
2			Craniofacial Science Laboratory, PPSG, USM	Active

 # Please attach the Asset/Inventory Return Form (Borang Penyerahan Aset/Inventori) – Appendix 1

D **RESEARCH ACHIEVEMENTS**

i **Project Objectives** *(as stated/approved in the project proposal)*

No.	Project Objectives	Achievement
1	To assess the attachment, spreading and growth of stem cells seeded on human AM; with/ without VEGF and sea cucumber extract treatment using scanning electron microscope (SEM)	Completed
2	To determine the proliferation rate of the seeded stem cells on AM, with/ without VEGF and sea cucumber extract treatment using Presto blue assay	Completed
3	To investigate the infiltration/ migration of the stem cells across the AM, with/ without VEGF and sea cucumber extract treatment using transmission electron microscope (TEM)	Completed (TEM method had been replaced with H&E method)
4	To determine the protein expression level of endothelial-cell markers of stem cells seeded on AM; with/ without VEGF and sea cucumber extract treatment by Western blotting	85% Completed
5	To determine the angiogenic gene expression levels of stem cells seeded on AM; with/ without VEGF and sea cucumber extract treatment by real time PCR	Completed (Method had been replaced with RT-PCR. Real time experiment is on going)
6	To compare the angiogenic effect between VEGF to sea cucumber extract during the stem cell angiogenesis differentiation	Completed

ii **Research Output**

a) Publications in ISI Web of Science/Scopus

No.	Publication (authors,title,journal,year,volume,pages,etc.)	Status of Publication (published/accepted/ under review)
1	Siti Nurnasihah Md Hashim, Muhammad Fuad Hilmi Yusof, Wafa' Zahari, Khairul Bariah Ahmad Amin Noordin, Thirumulu Ponnuraj Kannan, Suzina Sheikh Abdul Hamid, Khairani Idah Mokhtar & Azlina Ahmad (2016). Angiogenic Potential of Extracellular Matrix of Human Amniotic Membrane. *Korean Tissue Engineering and Regenerative Medicine*. Volume 13, Issue 3, pp 211–217. ISI indexed - Current impact factor – 0.941. First Online: 09 June 2016. DOI: 10.1007/s13770-016-9057-6.	Published
2	Md Hashim SN, Yusof MFH, Alshehadat SA, Kannan TP, Suzina SAH, Mokhtar KI & Azlina A. (2015). Morphological Change of SHED and the Effect Of VEGF on Amniotic Membrane Scaffold. *Malaysian Journal of Microscopy*. Vol. 11: 1-6. ISSN: 1823-7010. SCOPUS Indexed.	Published

3	Muhammad Fuad Hilmi Yusof, Wafa' Zahari, Siti Nurnasihah Md Hashim, Zul Faizuddin Osman, Hamshawagini Chandra, Thirumulu Ponnuraj Kannan, Khairul Bariah Ahmad Amin Noordin & Azlina Ahmad. Angiogenic and Osteogenic Potentials of Dental Stem Cells in Bone Tissue Engineering.	Submitted
4	Siti Nurnasihah Md Hashim, Tan Well Soon, Nurzulika Aqilah Md Aziz, Muhammad Fuad Hilmi Yusof, Khairul Bariah Mohd Amin Noordin, Thirumulu Ponnuraj Kannan, Ridzwan Hashim & Azlina Ahmad. The effect of *Stichopus Horrens* crude extract on the osteogenic genes expression of human extracted deciduous teeth stem cells.	In preparation
5	NFAT regulation on tubular morphogenesis of dental stem cell.	In preparation
6	VEGF effect on morphological, histological and gene expression of SHED on human amniotic membrane.	In preparation
7	Role of MEK Pathway in the SHED Angiogenic Differentiation in 3D Environment of Human Amniotic Membrane and VEGF Treatment.	In preparation

b) Publications in Other Journals

No.	Publication (authors,title,journal,year,volume,pages,etc.)	Status of Publication (published/accepted/ under review)

c) Other Publications
(book,chapters in book,monograph,magazine,etc.)

No.	Publication (authors,title,journal,year,volume,pages,etc.)	Status of Publication (published/accepted/ under review)

d) Conference Proceeding

No.	Conference (conference name,date,place)	Title of Abstract/Article	Level (International/National)
1	Health and Life Sciences Postgraduate Conference 2014, 10-11th June 2014, Renaissance Hotel, Kota Bharu, Kelantan - Oral presentation.	Proliferative Effect of Stichopus Horrens Extract on Stem Cells from Human Exfoliated Deciduous Teeth -	National
2	19th National Conference on Medical and Health Sciences, 7-8th September 2014, School of Dental Science, USM - Poster presentation.	Effect of Stichopus Horrens extract on cell proliferation and gene stem cell markers of dental stem cells	National

3	2-4th December 2015, Avillion Hotel, Melaka - Oral presentation.	Morphological Change of SHED and the Effect Of VEGF on Amniotic Membrane Scaffold - 24th Scientific Conference of the Microscopy Society Malaysia (SCMSM) 2015	National
4	13th Student Scientific Conference 2015, 17th December 2015, School of Dental Science, USM – Oral presentation	The effect of *Stichopus Horrens* crude extract on the osteogenic genes expression of human extracted deciduous teeth stem cells	National
5	National Colloquium on Stem Cell Research 2016, 7-8th March 2016, Hotel Perdana, Kota Bharu, Kelantan - Oral presentation.	Effect of vascular endothelial growth factor on stem cell and angiogenic gene markers of stem cell from human extracted deciduous teeth	National
6	National Colloquium on Stem Cell Research 2016, 7-8th March 2016, Hotel Perdana, Kota Bharu, Kelantan – Oral presentation.	Expression of protein stem cell markers by stem cells from human extracted deciduous teeth at different passages	National

 # Please attach a full copy of the publication/proceeding listed above

iii **Other Research Ouput/Impact From This Project**
(patent, products, awards, copyright, external grant, networking, etc.)

1. Collaboration with Prof Ridzwan Hashim, International Islamic University of Malaysia (IIUM).
2. Recipient of Science and Technology Research Grant 2015 by Malaysia Toray Science Foundation (MTSF).
   Title: Molecular Regulation of NFAT Tubular Morphogenesis in Stem Cell Angiogenic Differentiation for Dental Pulp Regeneration.
3. Best SEM Micrograph (Life Science Category) in 24th Scientific Conference of the Microscopy Society Malaysia (SCMSM) 2015.
   Title: Stromal side of HAM scaffold aids in new blood vessel formation

E HUMAN CAPITAL DEVELOPMENT

a) Graduated Human Capital

Student	Nationality (No.)		Name
	National	International	
PhD			1. 2.
MSc			1. 2.
Undergraduate			1.

b) On-going Human Capital

Student	Nationality (No.)		Name
	National	International	
PhD	1		1. Muhammad Fuad Hilmi Yusof
MSc	1		1. Siti Nurnasihah Md Hashim
Undergraduate	2		1. Tan Well Soon 2. Nurzulika Aqilah Md Aziz

c) Others Human Capital

Student	Nationality (No.)		Name
	National	International	
Post Doctoral Fellow			1. 2.
Research Officer			1. 2.
Research Assistant	3		1. Faraain Binti Ahmad 2. Abdul Mutalib Bin Kasim 3. Siti Nurnasihah Md Hashim
Others (.....)			1. 2.

F COMPREHENSIVE TECHNICAL REPORT

Applicants are required to prepare a comprehensive technical report explaining the project. The following format should be used (this report must be attached separately):

- Introduction
- Objectives
- Methods
- Results
- Discussion
- Conclusion and Suggestion
- Acknowledgements
- References

G PROBLEMS/CONSTRAINTS/CHALLENGES IF ANY

(Please provide issues arising from the project and how they were resolved)

1. We have troubled in maintaining HAESC. This type of cell were difficult to be maintained. Therefore, we have excluded this part and focusing only on one type of stem cell differentiation which is SHED.
2. Besides, the RNA yield from our 3D model (SHED seeded on HAM scaffold with/without VEGF) is very low. This problem was probably because of the extracellular matrix presence on HAM scaffold. So, a lot of time needed to conduct the experiment to yield enough RNA.
3. Last obstacle is, the methodology part. Transmission Electron Microscopy (TEM) part had been replaced to Hematoxylin and Eosin (H&E) method. We managed to prepare the TEM sample until the sectioning part only. It was very difficult to view and magnify at the desired tissue region since the membrane has been cut into small pieces in a resin. During our first trial, we do not get the expected result. However, with H&E, the result reflect our hypothesis. SHED was able to penentrate into the stromal side of HAM.

H	RECOMMENDATION
	(Please provide recommendations that can be used to improve the delivery of information, grant management, guidelines and policy, etc.)

Project Leader's Signature:

.....

Name : DR AZLINA AHMAD

Date : 17/4/17

I	COMMENTS, IF ANY/ENDORSEMENT BY PTJ'S RESEARCH COMMITTEE

GOOD ACHIEVEMENT). PLEASE ENSURE COMPLETION OF STUDIES BY STUDENT).

.....

Signature and Stamp of Chairperson of PTJ's Evaluation Committee

Name : PROF. DR. ADAM HUSEIN
DEKAN
Pusat Pengajian Sains Pergigian
USM Kampus Kesihatan
16150 Kubang Kerian, Kelantan.

Date : 2014/17

.....

Signature and Stamp of Dean/ Director of PTJ

Name : PROF. DR. ADAM HUSEIN
DEKAN
Pusat Pengajian Sains Pergigian
USM Kampus Kesihatan
16150 Kubang Kerian, Kelantan.

Date : 2014/17.

UNIVERSITI SAINS MALAYSIA

RU GRANT
FINAL REPORT CHECKLIST

Please use this checklist to self-assess your report before submitting to RCMO.
Checklist should accompany the report.

NO.	ITEM	PLEASE CHECK (✓)		
		PI	JKPTJ	RCMO
1	Completed Final Report Form	✓		✓
2	Project Financial Account Statement (e-Statement)	✓		✓
3	Asset/Inventory Return Form *(Borang Penyerahan Aset/Inventori)*	✓		✓
4	A copy of the publications/proceedings listed in Section D(ii) (Research Output)	✓		✓
5	Comprehensive Technical Report	✓		✓
6	Other supporting documents, if any			
7	Project Leader's Signature	✓		✓
8	Endorsement of PTJ's Evaluation Committee	✓		✓
9	Endorsement of Dean/ Director of PTJ's	✓		✓

UNIVERSITI SAINS MALAYSIA

BORANG PENYERAHAN ASET / INVENTORI

==========

A. BUTIR PENYELIDIK

1. NAMA PENYELIDIK :DR AZLINA AHMAD
2. NO STAF :
3. PTJ :
4. KOD PROJEK :
5. TARIKH TAMAT PENYELIDIKAN :14 DISEMBER 2016

B. MAKLUMAT ASET / INVENTORI

BIL	KETERANGAN ASET	NO HARTA	NO. SIRI	HARGA (RM)
1.	TRANS-BLOT TURBO (BIO-RAD)	2AK00014899PPSG	-	18, 639.00
2.	3D ROCKER, 230V	-	13050092	1, 490.00

C. PERAKUAN PENYERAHAN

Saya dengan ini menyerahkan aset/ inventori seperti butiran B di atas kepada pihak Universiti:

.....

(DR AZLINA AHMAD) Tarikh: 17/4/17

D. PERAKUAN PENERIMAAN

Saya telah memeriksa dan menyemak setiap alatan dan didapati :

☒ Lengkap
☐ Rosak
☐ Hilang : Nyatakan.....
☐ Lain-lain : Nyatakan .....

Diperakukan Oleh :

NOOR SALWAH S. OMAR
PEGAWAI ASET
BHG. PENYELIDIKAN DAN INOVASI
PUSAT PENGURUSAN PENYELIDIKAN
UNIVERSITI SAINS MALAYSIA
[illegible]

.....
Tandatangan
Pegawai Aset PTJ

Nama :
Tarikh : 18/4/2017

***Nota :** Sesalinan borang yang telah lengkap perlulah dikemukakan kepada Unit Pengurusan Harta, Jabatan Bendahari dan Pejabat RCMO untuk tujuan rekod.

BUDGET & EXPENDITURE

Tarikh cetakan : 16/04/2017

USM UNIVERSITI SAINS MALAYSIA APEX™

JABATAN BENDAHARI

PENYATA PERBELANJAAN SEHINGGA 16 APRIL 2017

Tajuk projek ANGIOGENIC POTENTIAL OF DENTAL STEM CELLS SEEDED ON HUMAN AMNIOTIC MEMBRANE AS A SCAFFOLD FOR PULP TISSUE REGENERATION

Tempoh projek 15/12/2012 hingga 14/12/2016

Taraf projek TAMAT TEMPOH (LAPORAN BELUM DITERIMA)

Ahli AZLINA BINTI AHMAD (KETUA PROJEK)

KHAIRANI IDAH BINTI MOKHTAR @ MAKHTAR

SUZINA BT. SHEIKH ABDUL HAMID

Nombor akaun

Vot	Nama Vot	Peruntukan Projek	Perbelanjaan Terkumpul Sehingga Thn Lalu	Baki Peruntukan Tahun Lalu	Peruntukan Thn Semasa	Jumlah Peruntukan Thn Semasa	Tanggungan Semasa	Bayaran Thn Semasa	Jum Belanja Thn Semasa	Baki Projek
111	GAJI									
221	JALANAN & SARA HIDUP									
223	HUBUNGAN & UTILITI									
227	BEKALAN BAHAN LAIN	1								
228	PENYELENGGARAN KECIL									
229	KHIDMAT IKTISAS									
335	HARTA MODAL									
552	PERBELANJAAN LAIN									
Jumlah		2								

Penyata ini adalah cetakan komputer tiada tandatangan diperlukan

Penyata ini adalah dianggap tepat jika tiada maklumbalas dalam tempoh masa 14 hari dari tarikh penyata

RESEARCH OUTPUT/IMPACT

pISSN 1738-2696 · eISSN 2212-5469
http://dx.doi.org/10.1007/s13770-016-9057-6
REVIEW ARTICLE

TERM

Angiogenic Potential of Extracellular Matrix of Human Amniotic Membrane

Siti Nurnasihah Md Hashim[1], Muhammad Fuad Hilmi Yusof[1], Wafa' Zahari[1], Khairul Bariah Ahmad Amin Noordin[1], Thirumulu Ponnuraj Kannan[1,2], Suzina Sheikh Abdul Hamid[3], Khairani Idah Mokhtar[4], Azlina Ahmad[1]*

[1]School of Dental Sciences, Universiti Sains Malaysia, Kelantan, Malaysia
[2]Human Genome Centre, School of Medical Sciences, Universiti Sains Malaysia, Kelantan, Malaysia
[3]Tissue Bank, School of Medical Sciences, Universiti Sains Malaysia, Kelantan, Malaysia
[4]Kulliyyah of Dentistry, International Islamic University Malaysia, Pahang, Malaysia

Combination between tissue engineering and other fields has brought an innovation in the area of regenerative medicine which ultimate aims are to repair, improve, and produce a good tissue construct. The availability of many types of scaffold, both synthetically and naturally have developed into many outstanding end products that have achieved the general objective in tissue engineering. Interestingly, most of this scaffold emulates extracellular matrix (ECM) characteristics. Therefore, ECM component sparks an interest to be explored and manipulated. The ECM featured in human amniotic membrane (HAM) provides a suitable niche for the cells to adhere, grow, proliferate, migrate and differentiate, and could possibly contribute to the production of angiogenic micro-environment indirectly. Previously, HAM scaffold has been widely used to accelerate wound healing, treat bone related and ocular diseases, and involved in cardiovascular repair. Also, it has been used in the angiogenicity study, but with a different technical approach. In addition, both side of HAM could be used in cellularised and decellularised conditions depending on the objectives of a particular research. Therefore, it is of paramount importance to investigate the behavior of ECM components especially on the stromal side of HAM and further explore the angiogenic potential exhibited by this scaffold.
Tissue Eng Regen Med 2016;13(3):211-217

Key Words: Angiogenic; Human amniotic membrane; Extracellular matrix; Tissue engineering

BIOLOGICAL SCAFFOLD IN TISSUE ENGINEERING

Tissue engineering is a field that has gone through much advancement since 1990s. It started when the scientist began to expand the cells on a scaffold of the desired tissue region with the help of external inducer to re-engineer the tissue. Currently, the combination between tissue engineering and other fields such as cell biology, biomaterials and cell imaging have brought in a great transition in this field, even though the intention is still on improving and repairing tissue function [1]. Apart from the selection of scaffold, the types of cells and growth factors used need to be chosen correctly to ensure the success in tissue reconstruction [2,3].

Received: July 13, 2015
Revised: August 14, 2015
Accepted: August 24, 2015
***Corresponding author:** Azlina Ahmad, School of Dental Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia. Tel: 609-767 5827, Fax: 609-767 5505, E-mail: azlinakb@usm.my

The selection of the best scaffold is important and need to be synchronised with the end target of the research. Basically, scaffolds which are also known as matrices, should be able to improve, repair and regenerate tissue function caused by injury, inflammation or disease [1,4,5]. A good scaffold should also provide a better environment for cell adhesion, proliferation and differentiation by allowing enough and efficient transportation of oxygen, nutrients and regulatory factors [1,6]. Besides, it needs to be biodegradable, biocompatible, and should provide structural integrity for both mechanical and physical strength [3,7]. Scaffold that provides a 3D micro-environment must also mimic the real conditions to enable the cell's function [3].

There are two types of scaffold, one made up of synthetic materials and the other, derived from a natural one. Examples of synthetic materials include poly(lactic acid), poly(glycolic acid) and polycaprolactone which are capable to sustain the growth of many types of stem cells due to their degradable fibrous structures [7]. Synthetic polymer has been shown to have a low possibility of contamination and readily to be engineered [8].

