



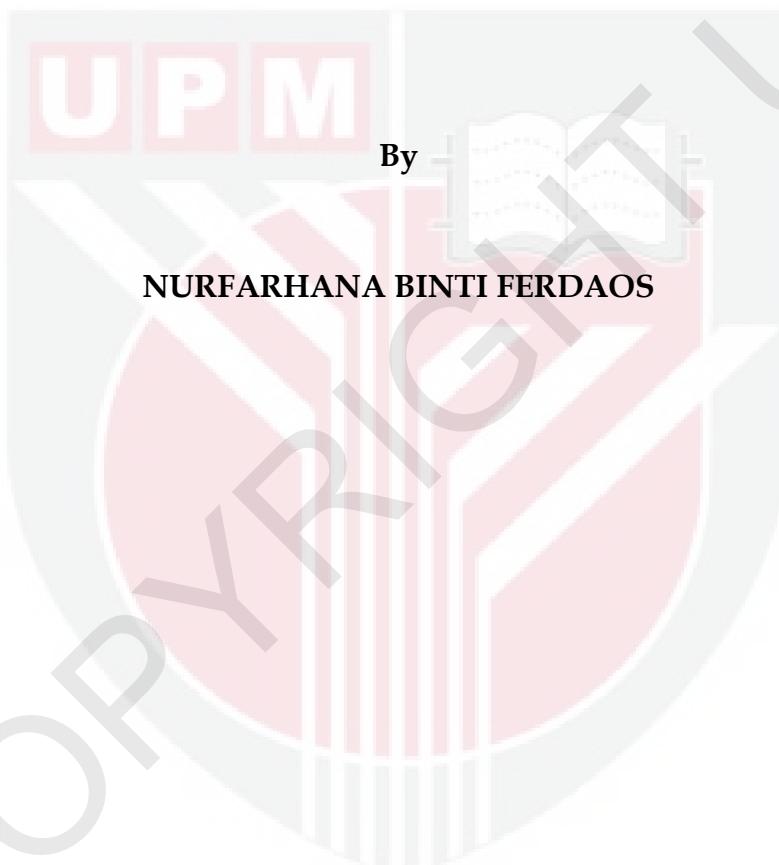
UNIVERSITI PUTRA MALAYSIA

**ISOLATION AND CHARACTERIZATION OF FULL-TERM AMNIOTIC
FLUID-DERIVED STEM CELLS**

NURFARHANA BINTI FERDAOIS

FPSK(m) 2010 20

**ISOLATION AND CHARACTERIZATION OF FULL-TERM AMNIOTIC
FLUID-DERIVED STEM CELLS**



**Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfilment of the Requirements for the Master of Science**

October 2010



Abstract of thesis presented on the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the Master of Science

**ISOLATION AND CHARACTERIZATION OF FULL-TERM AMNIOTIC
FLUID-DERIVED STEM CELLS**

By

NURFARHANA BINTI FERDAOS

October 2010

Chairman: Norshariza Nordin, PhD

Faculty: Medicine and Health Sciences

Stem cell research has gained many attentions for their hope to cure myriad of diseases ranging from genetically linked disorders to neurodegenerative diseases as well as injuries. Pluripotent stem cells signify the perpetual source of versatile cells, making them an excellent source for cell therapy. Current discoveries have reported amniotic fluid as an alternative source for pluripotent stem cells, termed amniotic fluid-derived stem (AFS) cells. AFS cells have been shown to express embryonic and adult stem cell markers while hindering the ethical controversies, which has been the biggest issue

involving the well-known pluripotent stem cells; the embryonic stem (ES) cells. In addition, AFS cells also do not form tumors upon transplantation. To date, only amniotic fluid of mid-term pregnancies has been reported to yield AFS cells. However, the collection of mid-term AF samples involves invasive procedure (amniocentesis) that may result in several complications including abortion. Therefore, in this study, we aim to isolate and characterize AFS cells from human and rat full-term pregnancies. The heterogeneity of amniotic fluid (AF) cells was observed upon culturing the cells under the optimized conditions, prior to isolation of stem cell by immunoselection against c-Kit, a marker for stem cell factor. The isolation of cells positive for c-Kit using Magnetic-Activated Cell Sorting (MACS) technology has resulted in enrichment of the c-Kit positive cells. Characterization of the c-Kit positive cells has demonstrated the expression of Oct-4, a marker for pluripotent cells and the formation of embryoid bodies (EBs), suggesting the possibility of AFS cells being present during full-term pregnancy in mammals including human. Hence giving hopes that full-term AFS cells would be the future alternative source for stem cell therapy.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**PEMENCILAN DAN PENCIRIAN SEL STEM CECAIR AMNIOTIK
DARIPADA TEMPOH PENUH KANDUNGAN**

Oleh

NURFARHANA BINTI FERDAOS

Oktober 2010

Pengerusi: Norshariza Nordin, PhD

Fakulti: Perubatan dan Sains Kesihatan

Penyelidikan dalam bidang sel stem telah menarik banyak perhatian kerana sel ini mempunyai potensi untuk menyembuhkan pelbagai jenis penyakit, merangkumi penyakit berkaitan genetik, sehingga penyakit berkaitan saraf termasuk kecederaan. Sel stem yang pluripoten adalah sumber semulajadi pelbagai jenis sel yang amat sesuai digunakan dalam terapi sel. Penemuan

terkini telah melaporkan bahawa cecair amniotik atau lebih dikenali sebagai air ketuban adalah sumber alternatif untuk sel stem yang pluripoten dan dinamakan sebagai sel stem cecair amniotik (AFS). Sel AFS telah terbukti berupaya mengekspres penanda sel stem untuk sel stem embrio (*embryonic stem cells*) dan sel stem dewasa (*adult stem cells*). Sel ini juga tidak mempunyai masalah etika yang kerap dikaitkan dengan sumber sel stem pluripoten yang diketahui umum iaitu sel stem embrio (*embryonic stem cells*). Tambahan pula, sel ini tidak membentuk ketumbuhan selepas proses transplantasi. Terkini, hanya air ketuban daripada kandungan tempoh pertengahan yang dilaporkan mengandungi sel AFS. Walaubagaimanapun, teknik untuk mengumpul air ketuban daripada kandungan tempoh pertengahan ini (amniosintesis) adalah berbahaya dan boleh menyebabkan beberapa komplikasi, termasuk keguguran. Oleh itu, projek ini bermatlamat untuk memencarkan dan mencirikan sel AFS daripada air ketuban dalam kandungan tempoh penuh manusia dan tikus. Kepelbagaiannya sel air ketuban telah didapati dalam tempoh pembiakan sel di bawah keadaan kultur sel yang optima. Proses pemenciran sel stem dijalankan dengan menggunakan proses pemilihan berdasarkan ekspresi ‘c-Kit’, iaitu penanda untuk faktor sel stem. Proses pemilihan ini yang menggunakan teknologi ‘*Magnetic-Activated Cell Sorting (MACS)*’ telah menghasilkan penambahan populasi sel yang positif untuk c-Kit. Pencirian sel yang positif untuk c-Kit menunjukkan ekspresi aktiviti Oct-4, iaitu penanda untuk sel pluripoten dan pembentukan ‘*embryoid bodies (EBs)*’. Penemuan ini mencadangkan terdapatnya sel AFS di

dalam air ketuban daripada kandungan tempoh penuh mamalia, termasuklah manusia. Oleh itu, sel AFS daripada kandungan tempoh penuh mempunyai harapan sebagai sel alternatif untuk terapi sel stem.

ACKNOWLEDGEMENTS

Praise be to Allah the Almighty, and peace be upon our prophet Mohammed. First and foremost, I must thank ALLAH S.W.T for His numerous blessings throughout my life and my journey to complete this thesis. Alhamdulillah.

My deepest thanks to my mentor and advisor, Dr. Norshariza Nordin for her invaluable support, guidance, and especially her patience throughout the years of my study. I really admire her strength and patience. Without her, I would not be able to finish this thesis. I would also like to thank Prof. Madya

Dr. Nazri for his expertise and time, in giving me amniotic fluid samples to carry out the project.

Special thanks to all the lecturers and friends in Medical Genetics Lab, for their helpful discussions and suggestions to improve my work. I would also like to thank UPM (RUGS grant) and MOHE (FRGS grant) for financial support.

Not to forget, my greatest appreciation to my parents and family. Words cannot say what love can do.

Thank you.

I certify that an Examination Committee has met on **16 November 2010** to conduct the final examination of **Nurfarhana binti Ferdaos** on her Master thesis entitled "**Isolation and Characterization of Full-Term Amniotic Fluid-derived Stem Cells**" in accordance with Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Master of Science.

Members of the Examination Committee were as follows:

Rozita Rosli, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Sabrina Sukardi, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Rajesh Ramasamy, PhD
Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

John Mason, PhD
Professor
Centre for Integrative Physiology
University of Edinburgh
Hugh Robson Building
George Square, Edinburgh EH8 9XD
United Kingdom
(External Examiner)

BUJANG KIM HUAT, PhD
Professor/ Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of **Master of Science**. The members of the Supervisory Committee were as follows:

Norshariza Nordin, PhD
Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Mohd Nazri Yazid, MD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)



HASANAH MOHD GHAZALI, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not

been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



NURFARHANA BINTI FERDAOS

Date: 11 October 2010

TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	v
ACKNOWLEDGEMENTS	vii
APPROVAL	viii
DECLARATION	x
LIST OF TABLES	xiv
LIST OF FIGURES	xv
LIST OF APPENDICES	xvii
LIST OF ABBREVIATIONS	xviii

CHAPTERS

1 INTRODUCTION	1
2 LITERATURE REVIEW	3
2.1 Introduction to stem cells	3
2.2 Characteristics of stem cells	4
2.2.1 Infinite self-renewal	4
2.2.2 Differentiation potential	5
2.3 Types of stem cells	7
2.3.1 Embryonic stem cells	7
2.3.2 Adult stem cells	9
2.3.3 Induced pluripotent stem (iPS) cells	12
2.3.4 Fetal stem cells	13
2.4 Amniotic fluid and the cell composition	14
2.4.1 The amniotic fluid	14
2.4.2 Cell populations within amniotic fluid (AF)	15
2.5 Stem cells in amniotic fluid	18
2.5.1 Amniotic fluid mesenchymal stem cells	18
2.5.2 Amniotic fluid-derived stem cells	20
2.6 Cell isolation techniques	21
2.6.1 Fluorescent activated cell sorting (FACS)	22
2.6.2 Magnetic activated cell sorting (MACS)	24
2.7 Characteristics of amniotic fluid-derived stem (AFS) cells	27
2.7.1 Cell morphology and growth	27
2.7.2 Immunophenotype of AFS cells	28
2.7.3 Differentiation potential of AFS cells	29
2.8 Advantages and future applications of AFS cells	32
2.8.1 <i>In vitro</i> applications	33
2.8.2 <i>In vivo</i> applications	34
3 MATERIALS AND METHODS	36
3.1 Methodology outline	36
3.2 Sample collection	36

3.2.1 Human AF sample collection	37
3.2.2 Rat AF sample collection	37
3.3 Culturing and maintenance of AF cells	38
3.4 Media optimization	39
3.4.1 Media preparation	39
3.4.2 Cell viability study	39
3.5 Cryopreservation and cells recovery	40
3.6 Isolation of c-Kit positive cells by using miniMACS	41
3.6.1 Preparation of degas buffer	41
3.6.2 Sample preparation and magnetic labeling	42
3.6.3 Magnetic separation	44
3.6.4 Elution of c-Kit positive cells	44
3.7 Media preparation for culturing of c-Kit positive cells	44
3.7.1 Chang medium	45
3.7.2 Embryonic stem (ES) cell medium	45
3.8 Culturing of AF c-Kit positive and negative cells	45
3.8.1 Preparation of 0.1% porcine gelatin	46
3.8.2 Preparation of mild trypsin	46
3.9 Flow cytometry	46
3.9.1 Preparation of washing buffers and solutions	47
3.9.2 Cell surface antigen staining	47
3.9.3 Intracellular antigen staining	48
3.9.4 Detection and analysis	48
3.10 Reverse-transcriptase polymerase chain reaction (RT-PCR)	49
3.10.1 RNA extraction and quantification	49
3.10.2 cDNA synthesis	50
3.10.3 Amplification of cDNA	51
3.10.4 Agarose gel electrophoresis	52
3.11 Spontaneous differentiation	52
3.11.1 Formation of multicellular aggregates (hanging drop method)	53
3.12 Immunocytochemistry (ICC)	53
4 RESULTS	56
4.1 Collection of amniotic fluid (AF) samples	56
4.1.1 Human AF samples	56
4.1.2 Rat AF samples	57
4.2 Media optimization for cultures of rat AF cells	58
4.3 Primary culture of AF cells	61
4.3.1 Human AF cells	61
4.3.2 Rat AF cells	63
4.4 Optimization of c-Kit primary antibody prior to immunoselection	66
4.5 Isolation of c-Kit positive cells	67
4.6 Optimization of culture conditions for c-Kit positive cells	71

4.7 Characterization of c-Kit positive cells	73
4.7.1 Molecular characterization	73
4.7.2 Telomerase activity	78
4.7.3 Spontaneous differentiation	79
5 DISCUSSION	86
5.1 Primary culture and viability of amniotic fluid cells	86
5.2 Amniotic fluid cells heterogeneity	91
5.3 Isolation of c-Kit positive cells	93
5.4 Characteristics of c-Kit positive cells	99
5.4.1 Cell morphology	99
5.4.2 Marker gene expression	100
5.4.3 Differentiation capacity	104
5.5 Limitations of the project	107
6 CONCLUSION AND FUTURE DIRECTION	110
6.1 Derivation of AFS cells from full-term pregnancies	110
6.2 Pluripotency of AFS cells	111
6.3 Potential applications of AFS cells	112
REFERENCES	114
APPENDICES	132
BIODATA OF STUDENT	153
LIST OF PUBLICATIONS AND PROCEEDINGS	154