# FABRICATION OF GRAPHENE OXIDE AND GRAPHENE-OXIDE/PEPTIDE BIOFILMS: CHARACTERIZATION, CELL VIABILITY AND DIFFERENTIATION POTENTIAL OF WHARTON'S JELLY MESENCHYMAL STEM CELLS

# **PUAH PERNG YANG**

# THESIS SUBMITTED IN FULFILLMENT FOR THE DEGREE OF MASTER OF SCIENCE

UNIVER

# FACULTY OF SCIENCE AND NATURAL RESOURCES UNIVERSITI MALAYSIA SABAH 2018

### **UNIVERSITI MALAYSIA SABAH**

#### BORANG PENGESAHAN TESIS

### JUDUL: FABRICATION OF GRAPHENE OXIDE AND GRAPHENE-OXIDE/PEPTIDE BIOFILMS: CHARACTERIZATION, CELL VIABILITY AND DIFFERENTIATION POTENTIAL OF WHARTON'S JELLY MESENCHYMAL STEM CELLS

### IJAZAH: MASTER OF SCIENCE (BIOTECHNOLOGY)

Saya **<u>PUAH PERNG YANG</u>**, sesi **<u>2014-2015</u>**, mengaku membenarkan tesis Sarjana ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:

- 1. Tesis ini adalah hak milik Universiti Malaysia Sabah.
- 2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
- 3. Perpustakaan dibenarkan membuat salinan tesisi ini sebagai bahan pertukaran antara institusi pengajian tinggi.
- 4. Sila tandakan (/):

**UNIVERSITI MALAYSIA SABAH** 

PETHIC

SULIT

(Mengandungi maklumat yang berdarjah keselamatan atau kepengtingan, Malaysia seperti yang termaktub di dalam AKTA RAHSIA 1972)



(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)



PÚAH PERNG YANG MS1421111T

Tarikh: 11 JUNE 2018

NURPERINGISMAIL SABAH (Tanda Tangan Pustakawan)

Assc. Prof. Dr. How Siew Eng Penyelia

### DECLARATION

I hereby declare that the material in this thesis is my own except for quotations, equations, summaries and references, which have been duly acknowledged.

26<sup>th</sup> MAY 2018

PUAH PERNG YANG MS 1421111T



### CERTIFICATION

NAME	:	PUAH PERNG YANG
MATRIC NO	:	MS1421111T
TITLE	;	FABRICATION OF GRAPHENE OXIDE AND GRAPHENE-
		OXIDE/PEPTIDE BIOFILMS: CHARACTERIZATION,
		CELL VIABILITY AND DIFFERENTIATION POTENTIAL
		OF WHARTON'S JELLY MESENCHYMAL STEM CELLS
DEGREE	:	MASTER OF SCIENCE
		(BIOTECHNOLOGY)
VIVA DATE	:	23 MAY 2018



**CO-SUPERVISOR** 

ASSC. PROF. DR. LEE PING CHIN

1h

#### ACKNOWLEDGEMENTS

I am deeply grateful to Grant TRGS0002-SG2/2014 for financially supporting this whole experimental work. I would like to give a special thank you to my supervisor, Assc. Prof. Dr. How Siew Eng and Assc. Prof. Lee Ping Chin for the continuous guide, proactiveness and support. The valuable time spent for discussion in teaching, planning and executing the experiment is truly invaluable for me to grow and evolve, learning not only about chemistry, biology and research, but the beauty of natural life!

Trillion thanks to the good Samaritans, who had helped in accomplishing this project and accompanying me through the thick and thin. Without each of them, nothing can be achieved. Firstly, Dr. Siti Fatimah and her student, Mis Warda, who helps in isolating and providing the Wharton's Jelly mesenchymal stem cell (WJ-MSCs) for my study.

Secondly, I shall remember Dr Moh Pak Yan for life as he spent countless hours in teaching me the preparation of biomaterials and their chemical characterizations. With his helping hand, understanding and analyzing AFM related data become easier and more sensible. Greatest respect to Dr. Teoh Peik Lin, who is willing to spend her precious time for meaningful discussion about problems related to cell culture work and screening experiment.

Next, the invaluable experience of Dr. Ling Yee Soon makes the lab work more interesting and adventurous. He taught me handling microscope skillfully and fine tuning my other lab skills. Not to forget Assc. Prof. Dr. Coswald, who is always positive and passionate about research work. His encouragement and experience sharing is always full of positive energy, inspiring and highly contagious.

Experimental work will not succeed without science officers, lab assistants and technicians, lab mates, both in chemistry and biology field, who always share their opinion and assist me in the laboratory work and empowering my skills and knowledge practically. Many thanks to Scihud's website for the providing an access to high impact and good quality journals as my scientific references. Last but not least, thanks to my family members who constantly support me throughout my master study, mentally and spiritually.

No words can express my gratefulness to those who have contributed and helped unexpectedly. Thank you for being part of my master journey, and for teaching me the real chemistry of biological LIFE. Without them, nothing is possible.

PUAH PERNG YANG 15<sup>TH</sup> SEPTEMBER 2018





### ABSTRACT

Human Wharton's jelly mesenchymal stem cells (WJ-MSCs) treatments are being tested clinically for a range of disorders. Surface modification techniques have been instrumental in the development of biomaterials that promote cellsurface interactions. In this study, the surface of graphene oxide (GO) was modified to promote the proliferation and differentiation of Wharton's jelly mesenchymal stem cells (WJ-MSCs). Synthesized GO was prepared through modified Hummers method, fabrication of GO film using drop-casting method and attachment of peptide to GO film through non-covalent approach,  $\pi$ - $\pi$ and electrostatic interactions. Synthesized GO were confirmed by UV-vis, XRD and FTIR. SEM and AFM images showed that synthesized GO has curled transparent thin film with thickness of 1.10 nm. Four peptide sequences, ("N"-YIGSRWYONMIRIKVAV-"C"), Pep2 ("N"namely Pep1 QHREDGSYIGSRIKVAV-"C"), Pep3 ("N"-WQPPRARIYIGSRIKVAV-"C") and Pep4 ("N"-DGEARGDSPKRSR-"C") were designed based short peptide sequences derived from extracellular matrix (ECM) adhesion peptides. AFM results revealed the thickness of GO biofilm (0.25 mg/mL) was 82.6 nm  $\pm$ 10.4 nm, corresponding to 65 - 85 layers of single layer GO. The GO biofilm (0.25 mg/mL) treated with Pep1 shows decrease in thickness as compared to non-treated GO film and the present of peptide bond in GO/Pep biofilm was confirmed by modified Lowry method. Furthermore, GO biofilms with concentration lower than 0.25 mg/mL were able to maintain the cell viability at day 5 as compared to glass coverslip. The WJ-MSCs were able to attach and growth on GO film. Increased of cell viability at day 6 was observed for all the GO/Pep biofilms as compared to GO biofilm. GO and GO/Pep biofilm allowed WJ-MSCs attachment, proliferation and increased in osteogenic differentiation capacity. Besides, the cell cultured on GO and GO/Pep biofilm able to maintain its undifferentiated stem cell characteristic. The data obtained here collectively demonstrates that the GO/Peptide biofilm assemble via noncovalent approach is a potential substrate for the adhesion, proliferation and enhance osteogenic differentiation of human WJ-MSCs. In conclusion, GO/Pep biofilms can be utilized for designing and manipulating biomaterials for stem cell, biological and tissue engineering applications.

#### ABSTRAK

### FABRIKASI BIOFILEM GRAPHENE OXIDE DAN GRAPHENE OXIDE/ PEPTIDA: PENCIRIAN, KEBOLEHAN HIDUPAN SEL DAN POTENSI PEMBEZAAN BAGI SEL STEM MESENKIMA WHARTON JELI

Rawatan sels stem mesenkima Wharton jeli manusia (WJ-MSCs) diuji secara klinikal untuk pelbagai penyakit. Teknik pengubahsuaian permukaan bagi menggalakkan interaksi sel dengan permukaan telah memainkan peranan penting dalam pembangunan biobahan. Dalam kajian ini, permukaan oksida grafit (GO) diubahsuai bagi menggalakkan kebolehan hidupan sel dan pembezaan WJ-MSCs. Oksida grafit yang disintesis melalui kaedah Hummers yang diubahsuai, fabrikasi filem GO dengan kaedah 'drop-casting' dan lekatan peptida ke filem GO melalui pendekatan bukan kovalen,  $\pi$ - $\pi$  dan interaksi elektrostatik. GO yang disintesis disahkan oleh UV-vis, XRD, dan FTIR. Imei SEM dan AFM menuniukkan bahawa GO vang disintesis mempunyai ketebalan 1.10 nm. Empat urutan peptida, Pep1 ("N"-YIGSRWYQNMIRIKVAV-"C"), Pep2 ("N"-QHREDGSYIGSRIKVAV-"C"), Pep3 ("N"-WQPPRARIYIGSRIKVAV-"C") dan Pep4 ("DGEARGDSPKRSR-"C") direka berdasarkan urutan pendek peptida dari matriks ekstraselular (ECM). Hasil AFM mendedahkan ketebalan GO biofilem (0.25 mg/mL) adalah 82.6 nm ± 10.4 nm, bersamaan dengan 65 - 85 lapisan tunggal GO. Biofilem GO (0.25 mg/mL) yang dirawat dengan Pep1 telah menunjukkan penurunan dalam ketebalan berbanding filem GO yang tidak dirawat, dan kemunculan ikatan peptida dalam GO/Pep biofilem telah disahkan oleh kaedah Lowry yang diubah suai. Tambahan pula, biofilem GO dengan kepekatan lebih rendah daripada 0.25 mg/mL tidak menunjukkan ketara dalam daya tahan sel pada hari ke-5, berbanding dengan kawalan (kaca). WJ-MSCs dapat melampirkan dan berkembang pada filem GO. Peningkatan daya maju sel pada hari 6 diperhatikan bagi semua biofilem GO/Pep, berbanding dengan biofilem GO. GO dan GO / Pep biofilem membenarkan lekatan WJ-MSC, kebolehan hiduoan sel dan peningkatan keupayaan pembezaan osteogenik. Selain itu, sel yang berbudaya pada biofilem GO dan GO / Pep dapat mengekalkan ciri sel stem yang tidak dibezakan. Data yang diperoleh di sini secara kolektif telah menunjukkan bahawa biofilem GO/Pep dihasilkan melalui pendekatan bukan kovalen adalah substrat yang berpotensi untuk melekatkan, mempercepatkan dan meningkatkan pembezaan osteogenik WJ-MSC manusia. Kesimpulanya, biofilem GO/Pep boleh digunakan dalam mereka bentuk dan memanipulasi biobahan untuk aplikasi stem sel, biologi dan tisu.

# TABLE OF CONTENTS

TITLE	Page i
DECLARATION	ii
CERTIFICATION	iii
ACKNOWLEDGEMENT	iv
ABSTRACT	vi
ABSTRAK	vii
TABLE OF CONTENTS	viii
LIST OF TABLES	xi
LIST OF FIGURES	xii
LIST OF SYMBOLS AND ABBREVIATIONS	xv
LIST OF APPENDICES	xvi
CHAPTER 1: INTRODUCTION	1
1.1 Research Background	1
1.2 Objectives	4
1.3 Scope of Work	4
UNIVERSITI MALAYSIA SABAH	
CHAPTER 2: LITERATURE REVIEW	5
2.1 Stem Cell	5
2.2 Wharton's Jelly Mesenchymal Stem Cells (WJ-MSCs)	7
2.2.1 WJ-MSCs as the Alternative Source of Mesenchymal Stem Cells	7
2.2.2 WJ-MSCs Differentiation Potential	9
2.3 Biomimetic Peptides as alternative to Extracellular Matrix (ECM)	11
2.4 Graphene-based biomaterials	14
2.5 Graphene oxide (GO) as biomaterials for Stem Cells	18
2.5.1 Method of GO Synthesis	18
2.5.2 Fabrication of GO Thin Film	20
2.6 Covalent and non-covalent binding of Graphene Oxide with peptides	22
2.6.1 Covalent binding of Graphene Oxide with peptides	22
2.6.2 Non-Covalent binding of Graphene Oxide with peptides	22

CHAPTER 3: METHODOLOGY	25
3.1 Synthesis of Graphene Oxide	25
3.2 Designation of Peptide Sequences	26
3.3 Fabrication of GO Thin Film	27
3.4 Fabrication of GO/Peptide Films	29
3.5 Characterization of GO flake, GO film and GO/Pep films	29
3.5.1 Phase identification of GO Flake	29
3.5.2 Determination of Functional Group of GO flake	30
3.5.3 Determination of Absorption spectra of GO flake	30
3.5.4 Determination of Morphology of GO, GO film and GO/Pep films	31
3.5.5 Determination of Thickness, lateral size and surface roughness	31
of GO, GO film and GO/Pep films	
3.5.6 Detection of Peptide on GO/Peptide film by modified Lowry's	31
Method	
3.6 Cell Culture Maintenance of Human Wharton's Jelly Mesenchymal	32
Stem Cells (WJ-MSCs)	
3.7 Cell culture in biomaterials	33
3.7.1 Cell Viability Assay	34
3.8 Cell Morphology	34
3.8.1 Observation of Cell Morphology by Phase Contrast Microscope	34
3.8.2 Observation of Cell Morphology by Scanning Electron	35
Microscope (SEM)	
3.9 In vitro Differentiation	35
3.9.1 Osteogenic Differentiation	35
3.9.2 Adipogenic Differentiation	36
3.10 Statistical analysis	36
CHAPTER 4: RESULTS AND DISCUSSIONS	37
4.1 Synthesized Graphene Oxide (GO)	37
4.2 Characterization of GO Flake	38
4.2.1 Phase identification of GO Flake	38
4.2.2 Thickness and lateral size of GO flake	38
4.2.3 Morphology of GO flake	40
4.2.4 Functional Group of GO flake	41

ix

4.2.5 Absorption spectra of GO flake	42
4.3 Characterization of GO Film and GO/Peptide Film	43
4.3.1 Morphology of GO Film and GO/Peptide Film	43
4.3.2 Surface roughness and thickness of GO Film and GO/Peptide	44
Film	
4.3.3 Detection of Peptide on GO/Peptide film by modified Lowry's	47
Method	
4.4 Cell culture of WJ-MSCs on GO biofilms	49
4.4.1 WJ-MSCs viability on the GO biofilms	49
4.4.2 WJ-MSCs morphology on the GO biofilms	50
4.5 Cell culture of WJ-MSCs on GO/Pep biofilms	54
4.5.1 WJ-MSCs viability and proliferation on the GO biofilms	54
4.5.2 WJ-MSCs morphology on the GO/Pep biofilms	56
4.6 Multipotent differentiation potential of WJ-MSCs on the GO/Pep	60
biofilms	
4.6.1 Osteogenic differentiation	61
4.6.2 Adipogenic differentiation	63
CHAPTER 5: CONCLUSION AND FUTURE STUDIES	65
REFERENCES	66
	89

# LIST OF TABLES

		Page
Table 2.1:	Selective synthetic peptide sequences of ECM proteins used in tissue engineering application.	13
Table 2.2:	Graphene-family substrates used in stem cell culture.	17
Table 3.1:	Characteristic of designed Peptide Sequences.	27
Table 3.2:	Related chemical reagents used in the peptide measurement.	32



## LIST OF FIGURES

		Page
Figure 2.1:	Wharton's jelly compartment of umbilical cord where W1-MSCs can be isolated.	8
Figure 2.2:	Registered clinical trials of human umbilical cord MSC from 2009- Jan of 2016. Total number of 109 trials was shown in the chart.	9
Figure 2.3:	Structure of graphene-family nanomaterials. (A) Few-layered graphene, (B) Graphene nanosheet, (C) Graphene oxide, (D) Reduced graphene oxide.	15
Figure 2.4:	Represent the synthesis of GO starting with graphite flakes. Under different oxidized condition, the hydrophobic carbon material was recovered during the purification of 'improved GO', Hummer's GO (HGO), and Hummer's modified GO (HGO+) method.	20
Figure 2.5:	The process of pre-treatment of glass substrates by using piranha solution and followed by 3-APTES solution.	21
Figure 2.6:	Schematic illustration of modification of GO film with PLL via non-covalent binding.	23
Figure 3.1:	Schematic illustration of the synthesis of Graphene Oxide.	26
Figure 3.2:	Four types of designed peptide sequences combined from three short bioactive peptides.	27
Figure 3.3:	Fabrication of five different concentrations of GO film and blank glass coverslip as control.	28
Figure 3.4:	Schematic illustration of the fabrication of GO/Peptide film.	29
Figure 3.5:	Schematic illustration of the WJ-MSCs culture on the biomaterials.	34
Figure 4.1:	Images of water dispersions of (1 mg/mL) of graphite powder (left) and synthesized graphene oxide (right).	37
Figure 4.2:	XRD pattern of GO.	38
Figure 4.3:	Simultaneously acquired AFM images of synthesized GO. (a) Height mapping image. (b) Adhesive force mapping. (c) The corresponding height profile obtained from the white dashed line box section in (a), indicating the thickness of synthesized GO sheet. [Blue arrow: glass coverslip; Green arrow: Single Layer of GO; Yellow arrow: Double layer of GO]. Mapping size is $10 \ \mu m \times 10 \ \mu m$ .	40
Figure 4.4:	SEM images of the morphology of GO thin film on the glass substrates under magnification factor of 5,000×.	41
Figure 4.5:	FTIR spectrum of GO.	42
Figure 4.6:	UV-visible spectrum of GO.	43
Figure 4.7:	SEM images of the morphology of GO thin film (left) and GO/Peptide thin film (right) on the glass	44

substrates under magnification factor of 7,000×. The red arrows indicate wrinkle area.

- Figure 4.8: AFM height images (a) of GO (left) and GO/Peptide (right) films deposited on the ATPES-treated glass coverslip. From the height images, line roughness profile could be extracted for any inserted dot line. Examples are shown in (b), where the line roughness profile along the white dot line in (a) are shown. The three-dimensional topography for both films were revealed in images (c).
- Figure 4.9: AFM height images (a) of GO (left) and GO/Peptide (right) films deposited on the ATPES-treated glass coverslip. From the height images, the thickness were measured by line across the substrate's film to surface non-coated (glass surface). The corresponding thickness profile could be extracted for any inserted line. Examples are shown in (b), where the line profile along the white line in (a) are shown.
- Figure 4.10: Concentration of Pep1, Pep2, Pep3 and Pep4 adsorbed onto varying concentration of GO films.
- Figure 4.11: Cell viability of the WJ-MSCs evaluated at different concentrations of GO films was quantified by MTT assay. WJ-MSCs were seeded in 48-well plate at a density of 1 x 104 cells/well with different concentrations of GO film and glass coverslip (control) for 5 days. Asterisk (\*) indicates statistically significance compared to control glass coverslip (n =3, p < 0.05).
- Figure 4.12: High-magnification SEM images of WJ-MSCs on (a) glass coverslip and (b) GO film, 0.25 mg/mL, at Day 3. The white arrows indicate WJ-MSCs adhered on glass coverslip and red arrows indicate WJ-MSCs adhered on GO film. Blue star indicate scratches on GO film.
- Figure 4.13: Morphology of cultured WJ-MSCs onto varying concentrations of GO biofilms at Day 5. The red arrows indicate WJ-MSCs adhered on substrates. Scale bar represent 200 µm.
- Figure 4.14: Cell viability of the WJ-MSCs evaluated at different concentrations of GO and GO/Pep biofilms was quantified by MTT assay. WJ-MSCs were seeded in 48-well plate at a density of 1 x 104 cells/well with different concentrations of GO film and glass coverslip (control) for 6 days. Asterisk (\*) indicates statistically significance compared to control glass coverslip (n = 3, p < 0.05).
- Figure 4.15: Morphology of cultured WJ-MSCs onto varying concentrations of GO and GO/Pep biofilms at Day 1, 3 and 6. The red arrows indicate WJ-MSCs adhered on substrates. Scale bar represent 200 µm.

59

46

47

48

50

52

56

51

xiii

Figure 4.16:	SEM images of WJ-MSCs on GO and GO/Pep biofilm, 0.25 mg/mL, at Day 3. The red arrows indicate WJ- MSCs adhered on substrates. Scale bar represent	60
Figure 4.17:	Osteogenic differentiation of WJ-MSCs on glass coverslip GO film and GO/Pep film. Scale bar	62
Figure 4.18:	Adipogenic differentiation of WJ-MSCs on glass coverslip GO film and GO/Pep film. Scale bar represent 100 µm	64
Figure A:	UV-Vis absorption spectra of Peptides after reaction with the biuret-Folin reagent in the modified Lowry's method	102
Figure B:	Calibration curve of Peptide-1 (Pep1) at 750 nm after reaction with the biuret-Folin reagent in the modified	103
Figure C:	Calibration curve of Peptide-2 (Pep2) at 750 nm after reaction with the biuret-Folin reagent in the modified Lowry's method.	104
Figure D:	Calibration curve of Peptide-3 (Pep3) at 750 nm after reaction with the biuret-Folin reagent in the modified Lowry's method.	105
Figure E:	Calibration curve of Peptide-4 (Pep4) at 750 nm after reaction with the biuret-Folin reagent in the modified Lowry's method.	106

UNIVERSITI MALAYSIA SABAH

## LIST OF SYMBOLS AND ABBREVIATIONS

&	And
ADSCs	Adipose-derived stem cells
AFM	Atomic force microscopy
BM-MSCs	Bone marrow derived mesenchymal stem cell
CD	Cluster of differentiation
ESC	Embryonic stem cell
FTIR	Fourier transformed infrared spectroscopy
GO	Graphene oxide
GO/Pep	Graphene oxide/Peptide
hESCs	Human embryonic stem cells
hMSCs	Human mesenchymal stem cells
hUC-MSCs	Human umbilical cord mesenchymal stem cells
iPSCs	Induced pluripotent stem cells
mg	Milligram
MHC	Major histocompatibility complexes
mL	Milliliter
MSCs	Mesenchymal stem cells
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
n	number
nm J	Nanometer
NSCs	Neural stem cells
PCL	Polycaprolactone
Pep1	Peptide-1
Pep2	Peptide-2
Pep3	Peptide-3
Pep4	Peptide-4 UNIVERSITI MALAYSIA SABAH
PXRD	Powder X-ray diffractometer
SD	Standard deviation
SEM	Scanning electron microscopy
UV	ultraviolet
WJ-MSCs	Wharton's jelly mesenchymal stem cells
μm	micrometer

# LIST OF APPENDICES

		Page
Appendix A	Peptide sequence 1 (Pep1)	89
	Peptide sequence 2 (Pep2)	92
	Peptide sequence 3 (Pep3)	95
	Peptide sequence 4 (Pep4)	98
Appendix B	Approval Letter Ethics and Research Committee of University Malaysia Sabah	101
Appendix C	Calibration curve for Peptide with Lowry method	102



## **CHAPTER 1**

# INTRODUCTION

#### 1.1 Research background

Recently, stem cell researches are expand rapidly with the potential to develop tissues/cells for transplantation, therapeutic agents to treat diseases as well as study disease development from early stages. Stem cells are an attractive prospect for tissue engineering and regenerative medicine due to their self-renewal capacity and multilineage differentiation (Higuchi et al., 2012). Stem cell can be isolated from embryonic and adult stem cells. Embryonic stem cells (ESC) isolated from inner cell mass of a blastocyst are pluripotent (Bongso et al., 1994), which able to differentiate into all types of cells from three germ layers namely, the ectoderm (epidermal tissue & nerves), mesoderm (muscle, bone & blood) and endoderm (liver, pancreas, gastrointestinal tract & lungs) (Thomas et al., 2009). However, their clinical applications are limited by cellular immune rejection, tendency to form tumors and ethical issue (Edwards, 2007; Maitra et al., 2005; Šarić et al., 2008). Therefore, adult stem cells began to become a potential substitute source of stem cell. Mesenchymal stem cells derived from bone marrow show similar functions as ESC and at the same time without involving embryos destruction (Takahashi & Yamanaka, 2006). Unfortunately, when it comes to clinical applications, the bone morrow derived mesenchymal stem cell (BM-MSCs) also face challenges, such as genetic alterations occurring due to ageing, limitation of cell numbers, decreased growth and differentiation capacity changes and painful isolation process (Baksh et al., 2007; Mimeault et al., 2007; Mueller & Glowacki, 2001). Thus, extensive research have been done in proving mesenchymal stem cells isolated from human umbilical cordderived stem cells as a new potential source of stem cells(Jin et al., 2013; Nagamura-Inoue & He, 2014; Wang *et al.*, 2009; Yousefifard *et al.*, 2016).

Extensive investigations of Wharton's jelly mesenchymal stem cells (WJ-MSCs) as a potential source of stem cell have been done. WJ-MSCs shared several remarkable features that make these cells suitable as an alternative source of mesenchymal stem cells. WJ-MSCs have shown the differentiation potential of WJ-MSCs into adipogenic, osteogenic, chondrogenic, angiogenic, neurogenic and myogenic (Aristea et al., 2013; Iwona et al., 2013; Pires et al., 2014; Xu et al., 2017), high capacity of proliferation and no risk is associated with teratoma formation, do not elicit an immune reaction which may reduce the risks of rejection upon transplantation (Davies et al., 2017; Karahuseyinoglu et al., 2007; Kim et al., 2013a; Medicetty et al., 2004; Rachakatla et al., 2007; Troyer & Weiss, 2008; Zhou et al., 2011), abundance and easy accessibility during pregnancy from healthy mothers as they are discarded after delivery (Li et al., 2017; Nagamura-Inoue & He, 2014), noninvasive isolation process and ethically free from controversial issues concerning human embryos as the stem cells sources (Edwards, 2007; Nagamura-Inoue & He, 2014). Due to these beneficial properties, WJ-MSCs shows ideal for clinical practice and good potential for cell therapy. However, WJ-MSCs have shown poor osteogenic and chondrogenic differentiation potential as compared to BM-MSCs (Hsieh et al., 2010; Wang et al., 2009). Hence, WJ-MSCs have been shown to increase its differentiation capacity by culturing in nano-scaffold (Gauthaman et al., 2010; Hosseini et al., 2015; Inthanon et al., 2016). Despite the differences, future scientific and clinical application of WJ-MSCs will require a detailed understanding of the mechanisms and signals that maintain their undifferentiated or enhance their differentiation capacity.

The stem cell 'niche' refers to the specific microenvironment features that regulates the fate of stem cells (Fuchs *et al.*, 2004). The stem cell fate is manipulated by varying the niche, for example, by altering the signaling molecules, stromal support tissue with cell-cell interactions, integrins-mediated cell-matrix interaction with the surrounding ECM, biophysical stimuli (Brafman, 2013). Recently, graphene-based nanomaterials are being applied to tissue engineering and induction of cell differentiations for *in vitro* stem cell culture.

2

Graphene-based biomaterials are able to modulate stem cell behaviors by providing a unique physical framework which proven to be comparable to natural ECM (Garcia-Alegria *et al.*, 2016; Kim *et al.*, 2013b; Marcela *et al.*, 2015). Due to the surface property of GO (hydrophilic) with the presence of oxygenated group enable them to bind with serum protein through electrostatic interactions and act as a pre-concentration platform to induce osteogenic and adipogenic differentiation (Lee *et al.*, 2011). Development of functionalized graphene oxide biomaterials is also necessary to improve their solubility and biocompatibility, and reduced cytotoxicity and genotoxicity in cellular application (Guo & Mei, 2014). The characteristic and biocompatibility of graphene-based nanomaterials may be controlled through surface functionalization either through covalent conjugation or noncovalent physisorption with other protein and peptides (Lu *et al.*, 2010; Sasidharan *et al.*, 2011).

To mimic the microenvironment of stem cell culture condition, extracellular matrix (ECM) components in important to enhance the growth and control stem cell fate (Ahmed & ffrench-Constant, 2016) Thus, synthetic biomaterials, such as peptides and polymers, are easily to fabricate and represent a reliable alternative for in vitro stem cell culture. Synthetic approaches for peptide materials, for example, short chain of amino acids, are quite different from protein materials, for example, long chains of amino acids. However, both strategies allow researchers the unique ability to program the exact monomer sequence within the biopolymer. Utilization of synthetic peptides as a component within engineered biomaterials is now a standard technique. For instances, peptides have been grafted to a variety of synthetic polymers to endow the material with cell-adhesive, enzymatically degradable and growth factor-binding properties.

In this study, GO biofilm were fabricated in various concentration and treated with bioactive peptide sequences via non-covalent approach as a biomaterials for culture WJ-MSCs. Then, direct contact of WJ-MSCs with various concentration of GO biofilm for certain cultured period via cell viability assay and morphological changes of cells were determined. The effect of bioactive peptide grafted on GO biofilm at different time point via cell morphology, cell viability assay and also the differentiation potential towards osteogenic and adipogenic lineages were studied.

3

### 1.2 Objectives

The objectives of this research are:

- To synthesize and characterize GO flakes, GO biofilms and GO/Peptides biofilms.
- (ii) To evaluate the cell viability of various concentrations of GO biofilms and proliferation potential of GO/Peptide biofilm on Wharton Jelly's mesenchymal stem cells (WJ-MSCs).
- (iii) To investigate the osteogenic and adipogenic differentiation potential of GO and GO/Peptides biofilms on WJ-MSCs.

#### **1.3 Scope of Work**

This research focus on the fabrication of biocompatible substrate for growing Wharton's Jelly mesenchymal stem cells (WJ-MSCs). Graphene oxide (GO) flake is first synthesize through the modified Hummers method from graphite flake. The fabrication of GO biofilms with varying ratio is prepare by drop-casted the synthesize GO solution on APTES-treated glass coverslip. For the fabrication of GO/Pep biofilm, peptide sequences is first design based on the bio-active short peptides. Then, GO/Peptide biofilms are prepare by assembly the different peptide sequences onto the surface of GO biofilm using non-covalent method. The surface of GO and GO/Pep biofilms are characterize using scanning-electron microscope (SEM) and atomic force microscope (AFM). The present of peptide bond on GO/Pep biofilm is measure using modified Lowry's method and Fourier transform infrared spectroscopy (FTIR). The cell viability of varying concentrations of GO biofilms are studied by culture with WJ-MSCs for 5 days using MTT assay. The osteogenic and adipospenic differentiation potential of both GO and GO/Pep biofilms are also studied with culture WJ-MSCs. The main objective is to evaluate the cell viability and differentiation potential of WJ-MSCs growth on the GO film before and after treated with peptide using non-covalent approach. The expected outcome and hypothesis is that the GO biofilm treated with peptide will allow better stem cell proliferation and increase the differentiation capacity.

### **CHAPTER 2**

# LITERATURE REVIEW

#### 2.1 Stem Cell

Stem cells are defined as cells that have two main characteristic: capability to proliferate indefinitely and multilineage differentiation (Leeb et al., 2010; Zhang & Fu, 2008). These properties of human stem cells have been extensively studied for their regeneration processes, cell-replacement therapies, use for disease modeling and drug screening (Srivastava & Ivey, 2006; Wu & Izpisua Belmonte, 2016). Basically, stem cell can be classified based the differentiation capacity. Stem cell produced from the fusion of an egg and sperm cell are totipotent cells which is the most important stem cells that capable to differentiate into all types of cell, including cell for development of placenta, umbilical cord, and any other cell found in the adult body (Baker & Pera, 2018; Forraz & McGuckin, 2011). Embryonic stem cells isolated from inner cell mass of a blastocyst are pluripotent cells and potential to give rise to any differentiated cell in the body, except the placenta and umbilical cord (Hima & Srilatha, 2011; Thomson et al., 1998). Induced pluripotent stem cells (iPSC) discovered by Yamanaka and coworkers by inducing the somatic terminally differentiated cell to express a number of genes that are normally present in embryonic stem cell to produce stable lines of embryonic-like pluripotent stem cells (Takahashi et al., 2007; Takahashi & Yamanaka, 2006). Adult or somatic stem cells can also be isolated from various adult human tissues, such as brain, bone marrow, skin, liver, gut, fat and more. These adult stem cells can be isolated from several tissue sources, such as bone marrow, the central nervous system and skeletal. They are multipotent stem cells which can proliferate and differentiate into those closely related family of cells (Toma et al., 2001). In the context of clinical applications of stem cells, totipotent stem cells, pluripotent stem cells (embryonic stem cell) and iPSC are currently constrained by cellular immune rejection (Sarić et al., 2008),

tendency to form tumors (Maitra *et al.*, 2005; Shih *et al.*, 2011), ethical issue concerning human embryos as the sources (Edwards, 2007; Nagamura-Inoue & He, 2014) and low efficiency induced gene expression (for iPSC) (Forraz & McGuckin, 2011; Yamanaka, 2012). In contrast, the use of adult stem cells such as mesenchymal stem cells does not involve destruction of human embryos and at the same time show similar functions as ESC (Okolicsanyi *et al.*, 2015; Takahashi & Yamanaka, 2006). Adult stem cells have been used for many years for leukemia and blood/bone cancers treatment though bone marrow transplants (Moorthy, 2011; Radhika & Laxmi, 2011).

Mesenchymal stem cells (MSC) are adult stem cells that can proliferate as undifferentiated cells and able to potentially differentiate to lineages of mesenchymal tissues, such as cartilage, bone, tendon, fat, muscle and marrow stroma (Pittenger et al., 1999) (Okolicsanyi et al., 2015). Mesenchymal stem cell can be isolated mainly from adult tissues, including bone marrow (Wexler et al., 2003) and adipose tissue (Zuk et al., 2002). To a lesser extent, MSC can also isolated from dental pulp (Gronthos et al., 2000), trabecular bone (Nöth et al., 2002), tendon (Bi et al., 2007), placenta (Fukuchi et al., 2004), umbilical cord (Romanov et al., 2003), amniotic fluid, synovia (De Bari et al., 2001) and others. Due to the lack of standard isolation, culture and characterizing protocols, results in many difficulties when comparing the study outcomes. Thus, in year 2006, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) proposes minimal criteria to define human mesenchymal stem cells (hMSCs). Firstly, hMSCs must be plastic-adherent in classical culture conditions. Secondly, under specific in vitro differentiation conditions, hMSCs must be able to consistently differentiate into trilineage differentiation, including adipocytes, osteoblasts and chrondroblasts. Additional requirements included hMSC population must express high levels ( $\geq$  95%) positive) of CD105, CD73 and CD90, and the low expression ( $\leq 2\%$  positive) of the hematopoietic markers, CD 34, CD11b or CD14, CD 45, CD19 or CD79a and HLADR (Dominici et al., 2006; Keating, 2012). For multipotentcy plastic adherent cells with lack of characterization data, the term 'multipotent mesenchymal stromal cell' with the same acronym MSC can be used to indicate these unique properties without ascribing homogeneity or stem cell activity (Davies et al., 2017; Horwitz et al., 2005).

For the past few years, clinical application of autologous bone marrow mesenchymal stem cells (BM-MSCs) was reported for conditions, including Crohn's disease (Duijvestein *et al.*, 2010), cardiac infarction (Minguell *et al.*, 2011), bone tissue engineering (Kagami *et al.*, 2011) and graft-versus-host disease (GVHD) (Muroi *et al.*, 2013; Muroi *et al.*, 2016; Weng *et al.*, 2010). Besides, BM-MSCs also face challenges like limitation of cell numbers, decreased growth and differentiation capacity due to age-related changes when it applied for clinical applications (Baksh *et al.*, 2007; Mueller & Glowacki, 2001). In recent years, extensive studies focus on human umbilical cord mesenchymal stem cells (hUC-MSCs) with similar gene expression profile of hESCs and faster self-renewal as compared to BM-MSCs as the alternative source of stem cell (Baksh *et al.*, 2007; Jin *et al.*, 2013; Malgieri *et al.*, 2010; Nagamura-Inoue & He, 2014; Wang *et al.*, 2009; Yousefifard *et al.*, 2016).

#### 2.2 Wharton's Jelly Mesenchymal Stem Cells (WJ-MSCs)

### 2.2.1 WJ-MSCs as the Alternative Source of Mesenchymal Stem Cells

Human umbilical cord is considered as medical waste has been collected and used as the alternative source of stem cells. Isolation of mesenchymal stem cell from umbilical cord is noninvasive and does not encounter ethical problems (Li *et al.*, 2017; Nagamura-Inoue & He, 2014). Human umbilical cord with an inner tissue contain two arteries and one vein and is surrounded by a connective tissue called Wharton's jelly (Figure 2.1). Mesenchymal stem cells which directly obtained from Wharton's jelly, and are known as human Wharton's jelly mesenchymal stem cells (WJ-MSCs) (Forraz & McGuckin, 2011). Fibroblast-like cells were first isolated about 20 years ago from Wharton's jelly (McElreavey *et al.*, 1991). In 2004, WJ-MSCs were finally proved to be MSCs, as the cells expressed CD 105, CD 73, CD 51, CD 44 and CD 29, lacked expression of CD 45 and CD 34, and proved to be able to differentiate into osteogenic and adipogenic lineages (Wang *et al.*, 2004). Currently, WJ-MSCs can be isolated either using the explant method or the enzymatic digestion method and most likely consist of a heterogeneous population (Seshareddy *et al.*, 2008).