CARTILAGE TISSUE ENGINEERING: EXPLORING THE POTENTIAL OF POLY (LACTIC-CO-GLYCOLIC ACID) BASED SCAFFOLDS AND THE BIOETHICAL ASPECT FROM ISLAMIC PERSPECTIVE

BY

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A thesis submitted in fulfilment of the requirement for the degree of Doctor of Philosophy in Health Sciences

Kulliyyah of Allied Health Sciences
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MAY 2016

ABSTRACT

Treating articular cartilage defect remains a major orthopaedic predicament. Being a simple structure that is avascular and aneural, articular cartilage has little ability to repair itself when damaged. Cartilage tissue engineering provides an alternative technique for restoring and regenerating damaged cartilage tissues. This study attempts to (1) evaluate and compare the growth of bone marrow mesenchymal stem cells (BMSCs) aspirated from rabbits' iliac crest and femur in two different culture media; α-Minimum Essential Medium (α-MEM) and a mixture of 1:1 Ham's F12 Nutrient Mixture (F12) and Dulbecco's Modified Eagle Medium (DMEM) or FD, (2) to evaluate the *in vitro* and *in vivo* cartilaginous tissue formation using BMSCs-seeded poly(lactic-co-glycolic acid) PLGA/Fibrin scaffolds and (3) to explore the bioethical issues within the Islamic perspective in relation to tissue engineering and regenerative medicine (TERM). Growth kinetic assessments were performed on monolayer-cultured BMSCs harvested from two locations and cultured in two different media. Constructs fabricated from BMSCs seeded onto PLGA/Fibrin were cultured for three weeks and thereafter were implanted in rabbit model. The *in vitro* cartilaginous engineered constructs were evaluated by gross inspection, cell proliferation assay, gene expression, sulphated glycosaminoglycan (sGAG) production and histology at week 1, 2 and 3 while the *in vivo* construct were harvested at week 6 and week 12 post-implantation. It was observed that BMSCs harvested from the iliac crest region and cultured in FD significantly promoted BMSCs growth. The *in vitro* study showed that after 3 weeks of culture, the PLGA/Fibrin construct exhibited significantly higher cell viability, higher sGAG content and better histo-architecture and cartilaginous extracellular matrix (ECM) compound in concert with the positive glycosaminoglycan accumulation when compared to the PLGA only construct. Post implantation, the osteochondral defects treated with PLGA/Fibrin/BMSCs constructs showed better repair, more cartilaginous extracellular matrix, higher sGAG content, superior compressive strength and greater expression of chondrogenic marker genes than PLGA/BMSCs group. *COL2A1* which is the specific cartilage marker, *ACAN*, *COL9A1* and *SOX9* genes were expressed both in the *in vitro* and *in vivo.* This study suggested that PLGA/Fibrin when seeded with pluripotent BMSCs that underwent optimum manipulation may serve as a prospective construct to be developed as functional tissue engineered cartilage. This study may also act as a platform to spark the initiative in integrating Islamic bioethics in providing "middle of the road" approach moderating between science and religion. Debates on TERM must comprise on religious and ethical view considering there are still many grey areas that requires Islamic input. Based from the Islamic perspective, scientific researches in TERM are permitted, as long as it is not a threat to human being and applied within the permissible limits described by the *Shari'ah.*

خلاصة البحث

علاج خلل الغضروف المفصلي يبقى مأزقاً كبيراً للعظام. وكونه هيكلاً بسيطاً , مع عدم وجود النسيج العصبي وعدم ارتباطه بالأوعية الدموية فالغضروف المفصلي لديها القليل من القدرة على إصلاح نفسه عندالتلف. وتقدم هندسة أو تقنية الأنسجة الغضروف تقنية بديلة لاستعادة وتجديد أنسجة الغضروف التالفة. وتحاول هذه الدراسة (1) تقييم ومقارنة نمو الخلايا الجذعية نخاع العظام الوسيطة (BMSCs) المستنشق من عرف الحرقفة الأرانب وعظم الفخذ منها في وسيلتي الثقافة المختلفتين. α-الدنيا الأساسية المتوسطة (α-MEM) وخليط من 1: 1 هام F12 المغذيات خليط (F12) وDulbecco لتعديل النسر المتوسطة (DMEM) أو FD، (2) لتقييم في المختبر والمجراة تكوين الأنسجة الغضروفية باستخدام بولي (حمض اللبنيك، شارك في الجليكوليك) السقالات PLGA / الليفين المصنف بـBMSCs و (3) لاستكشاف قضايا أخلاقيات العلوم الحيوية في وجهات النظر الإسلامية فيما يتعلق بهندسة الأنسجة والطب التجديدي (TERM). أجريت النمو التقييمات الحركية على المثقفة BMSC ذات الطبقة الواحدة التي تحصد من موقعين ومثقف في وسيلتي الثقافة المختلفتين. المصنفة المبنية من BMSCs ملفقة على PLGA / الليفين كانت تثقف لمدة ثلاثة أسابيع وبعد ذلك تم زرعها في نموذج أرنب. وقد تمّ في المختبر تقييم غضروفي المبنى بالتفتيش الإجمالي، تكاثر الخلايا الفحص، التعبير الجيني، إنتاج غلكسمينوغلكن المكبرت (sGAG) والأنسجة في الاسبوع 1 و 2 و 3 في حين تم حصادها في الجسم الحي يبني في الأسبوع 6 و 12 أسبوع بعد الزرع . ولوحظ أن BMSCs المحصود من المنطقة عرف الحرقفة والمثقف في FD عززت بشكل كبير نمو BMSCs. كما أظهرت دراسة في المختبر أنه بعد 3 أسابيع من الزرع، وبنيات PLGA / الليفين أظهرت أعلى بقاء الخلية بكثير، وارتفاع محتوى sGAG وأفضل عمارة الأنسجة والغضروفية المصفوفة خارج الخلية (ECM) مجمع بالتنسيق مع تراكم جلايكان إيجابي بالمقارنة مع بناء PLGA فقط. وأظهرت آخر الزرع، والعيوب العظمية الغضروفية المتعامل مع بنيات PLGA / الليفين أفضل إصلاح، أكثر خارج الخلية الغضروفية المصفوفة، وارتفاع محتوى sGAG، قوة الضغط العالية وزيادة التعبير عن علامات الجينات المولدة للغضروف من مجموعة PLGA. وأعرب عن البروتين الأساسية aggrecan، نوع الكولاجين التاسع، جين sox9 والكولاجين النوع الثاني، الذي هو علامة الغضروف المحددة في كلٍ من المختبر والمجراة. واقترحت هذه الدراسة إلى أن PLGA / الليفين عند التصنيف مع BMSCs المحفزة التي خضعت التلاعب الأمثل قد يكون بمثابة بناء المحتملين يجري تطويرها لأنسجة وظيفية الغضروف المهندسة. كما يمكن أن تعمل هذه الدراسة أيضاً كمنصة لاشعال المبادرة في دمج أخلاقيات علوم الأحياء الإسلامية في توفير "منتصف الطريق" نهج الاعتدال بين العلوم والدين. يجب أن تكون المناقشات حول هندسة الأنسجة والطب التجديدي (TERM) تشمل على وجهة نظر الديني والأخلاقي إذ أنّ هناك الكثير من الوجوه التي تتطلب إدخال الإسلامي. وعلى أساس من المنظور الإسلامي، تسمح الأبحاث العلمية مثل هندسة الأنسجة والطب التجديدي (TERM)، طالما أنها لا تشكل تهديدا للإنسان وتمّ تطبيقها ضمن الحدود المسموح بها التي حدّدها الشريعة الإسلامية.

APPROVAL PAGE

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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I dedicate this thesis to my loving family for their continuous support and affection throughout my life.

ACKNOWLEDGEMENTS

Firstly, my utmost gratitude above all due to Allah s. w. t for His mercy and blessing I have been able to complete this thesis.

My deepest appreciation goes to my respected supervisor, Assist. Prof. Dr. Munirah Sha'ban for her continuous guidance, patience, advice and encouragement in spite of her tight schedule, which contributed to the completion of this work. Similar appreciations are expressed to my co-supervisors; Prof. Dr Ahmad Hafiz Zulkifly, Assoc. Prof. Dr. Aminudin Che Ahmad and Assoc. Prof. Dr. Abdurezak Abdulahi Hashi, for their support, idea and assistance throughout completing this work. My appreciation also goes to the International Islamic University Malaysia, particularly the Kulliyyah of Allied Health Sciences for providing me the opportunity to study and prepare this work. I acknowledge the Ministry of Higher Education Malaysia for granting the financial assistance to complete this project.

My cordial thanks also go to Br. Hanif and Kak Wan from the Histopathology lab at the Kulliyyah of Medicine for providing valuable assistance when I was preparing my slides. A special thank you note to my previous final year students; Aida Hayati, Norhafizah, Naily, Athirah, Aida Hanisa, Zaty and Fatin for their assistance and colourful memories. My deepest gratitude to my fellow comrades; Hidayah, Azam and Miza for their friendship, endless help, support and memorable lunch dates from the beginning of my journey. It was a great privilege to work with you guys who always motivated me during the time I was working on my thesis.

I am deeply indebted to my family, especially to my beloved parents Haji Abdul Rahman bin Haji Daud and Hajjah Wan Zaiton bt. Haji Wan Hitam who have never given up praying for their daughter from the very beginning of her life, and for all the 'how's your study going on?' phone calls, also to my dearest siblings Reena and Boy for their moral support and motivation. To my dear mother in-law Hajjah Zaliha Mahmud and my other extended families and friends, thank you from the bottom of my heart for your prayers.

Finally, I owe a very special thank you to my significant other half, Wan Azdie for his undying patience, understanding, support, love and of course his delicious home cook meals that never disappointed me, and to my beloved children Wan Muhammad Aiman and Wan Aysha Humaira, I seek their forgiveness for the times I was being over the edge and for not being able to spend much time and money while they were growing up.

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LIST OF ABBREVIATIONS

3-D	Three Dimensional
ACI	Autologous Chondrocyte Implantation
ASCs	Adult Stem Cells
B. C	Before Christ
bFGF	Basic Fibroblast Growth Factor
BMP	Bone Morphogenetic Protein
BMSCs	Bone Marrow Mesenchymal Stem Cells
$CaCl_2$	Calcium Chloride
cDNA	Complementary Deoxyribonucleic Acid
CH_2Cl_2	Methylene Chloride
CO_2	Carbon Dioxide
COX	Cyclooxygenase
DMSO	Dimethyl Sulfoxide
ECM	Extracellular Matrix
EDTA	Ethylenediamine Tetra Acetic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
ERGS	Exploratory Research Grant Scheme
ESCs	Embryonic Stem Cells
F	Femur
FBS	Foetal Bovine Serum
FD	Ham's F-12 Nutrient Mixture and Dulbecco's Modified Eagle Medium
FDA	Food and Drug Administration
GAG	Glycosaminoglycan

H&E	Haematoxylin and Eosin
H_2O_2	Hydrogen Peroxide
HEPES	4-(2-Hydroxyethyl)-1-Piperazineethanesulfonic Acid
IC	Iliac Crest
ICRS	International Cartilage Repair Society
IGF-1	Insulin-like Growth Factor-1
IL-1	Interleukin -1
IM	Intra-muscular
IQR	Interquartile Range
ITS	Insulin Transferrin Selenium
KTX	A combination of drugs Ketamine, Tilatamine and Xylazine for anaesthesia
MACI	Matrix Induced Autologous Chondrocytes Implantation
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
MSCs	Mesenchymal Stem Cells
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
Na_2HPO_4	Disodium Hydroxyphosphate
NaCl	Sodium Chloride
NSAIDs	Non Steroidal Anti-Inflammatory Drugs
NZW	New Zealand White Rabbit
OA	Osteoarthritis
OATS	Osteochondral Autologous Transplantation
P	Passage
PBS	Phosphate Buffered Saline
PBS-AA	Phosphate Buffered Saline-Antibiotic Antimycotic

PCL	Polycaprolactone
PGA	Poly-glycolic Acid
PLA	Poly-lactic Acid
PLGA	Poly(lactic-co-glycolic acid)
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction
S.E.M	Standard Error of Mean
SEM	Scanning Electron Microscopy
sGAG	Sulphated Glycosaminoglycan
SPSS	Statistical Package for Social Science
TEMPs	Tissue Engineered Medical Products
TERM	Tissue Engineering and Regenerative Medicine
TGF-β	Transforming Growth Factor Beta
TNF-α	Tumour Necrosis Factor-Alpha
WHO	World Health Organization
α-MEM	Alpha- Minimum Essential Media

LIST OF SYMBOLS

%	Percentage
®	Registered patent
°C	Degrees Celsius
µl	Microliter
µm	Micrometer
cm	Centimeter
g	Gram
IU	International Unit
kg	Kilogram
mg	Milligram
ml	Milliliter
mm	Millimeter
MPa	Mega Pascal
rpm	Revolutions per minute
™	Trademark

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Articular cartilage is a specialized connective tissue populated by highly specialized designed cells of mesenchymal origin. Being a unique structure, articular cartilage is known to have a very d capacity for self-repair. This is because it lacks blood vessels, lymphatic and nerve supply (Pearle, arren, & Rodeo, 2005). Hence, nutrients' supply and repair activities could not be done completely it is injured. Due to its poor blood supply and poor capacity of self renewal, treating damaged articular cartilage remains a challenging surgical procedure in the orthopedic world (Tanideh, Nazhvani, Jaberi, Mehrabani, & Rezazadeh, 2011).

The architecture and function of articular cartilage can be damaged with regular wear and tear which results from a bad fall or probably a traumatic sport injury. Most people treat joint pains as part and parcel of ageing and do not see the urgency to seek treatment immediately. However, after experiencing the agony of joint pains which affect their daily chores will they then consult a doctor. However, once articular cartilage is damaged and left untreated, the condition may lead to osteoarthritis (OA). Osteoarthritis is the most prevalent form of arthritis, affecting millions of people around the world. It happens when the joint cartilage breaks down frequently because of repeated mechanical stress over a period of time. With age, the protective cartilage which covers the bony surface and acts as a cushion for weight bearing, wears down and expose the underlying bone to direct pressure which later can progress into OA. In the Global Burden of Disease 2010 study, it was reported that approximately 251 million people suffered from knee OA worldwide (Murray et

al., 2013). Since OA is a degenerative disease, it is prevalent in the ageing population. The World Health Organization (WHO) had estimated that 524 million people were aged 65 or older in the year 2010. Fast forward 40 years to come, this number is expected to triple, amounting to 16% of the world's population by 2050 (World Health Organization, 2011). In the midst of evolving to be a developed country, it was estimated that 13.1% of the Malaysian population were aged 55 or older in 2014 and the life expectancy has dramatically increased to 70 years or older (Index Mundi, 2014). Looking at this scenario, it is likely to say that Malaysia will reach an ageing nation status. As age is one of the risk factors for OA, the incidence of this condition will undoubtedly increase. According to Sockalingam, (2011) the number of OA cases in Malaysia is escalating and it was estimated that 60% of the population will suffer from some form of arthritis by the age of 60, with more than 20% of the cases involved OA (Lim, 2008). Indeed OA is considered as one of the major causes for morbidity around the world which has physical, emotional, social and economical pressure that could impinge upon one's daily life.

As there is no cure for OA yet, numerous trials of research and study involving drugs and surgical interventions have been done to treat damaged articular cartilage (Hauser, 2010; Malemud, 2013). Normally, drugs such as paracetamol, conventional non-steroidal anti-inflammatory drugs (NSAIDs), diacerein, glucosamine, and chondroitin sulphate are widely used to reduce the pain among osteoarthritis patients (Falah, Nierenberg, Soudry, Hayden, & Volpin, 2010). Patients with moderate to severe OA are given steroid and viscosupplementation injections (Stanos, 2013). In spite of that, non-surgical treatment options may only support short-term alleviation. In more severe cases, patients who do not respond to conservative treatment are subjected to surgery. These surgical interventions include osteochondral autograft

transplantation, mosaicplasty, microfractures and autologous chondrocytes implantation (ACI). Intervention mainly depends on the size of the lesion, age of the patient and availability of treatment (Rozlin , Nor Azlina, & Munirah, 2013). Although these techniques have varying success rates, some long-term follow-ups have reported unsatisfactory outcomes (Bentley et al., 2003; Jakob, Franz, Gautier, & Mainil-Varlet, 2002; Mithoefer, McAdams, Williams, Kreuz, & Mandelbaum, 2009; Seo et al., 2009). The gold standard in treating articular cartilage disease is to restore the joint surface with suitable tissues, provide pain relief and most importantly to put a halt in further joint degeneration. To date, it still remains a challenge for surgeons and researchers to create a replacement that bears a resemblance to the native cartilage in terms of its structure and composition.

Over the past few decades, articular cartilage restoration has witnessed a great deal of improvement in the area of tissue engineering. Made possible by Langer & Vacanti (1993), tissue engineering aims to repair, regenerate and restore the functional and mechanical properties of a native articular cartilage. It is rather intricate to say exactly when tissue engineering was first introduced because its principles have been applied since the ancient times (Kretlow & Mikos, 2010). Probably one of the earliest examples of tissue engineering was an ancient Egyptian text illustrating sutured closure of wounds for healing dated between 2600-2200 B.C. Around 600 B.C, the first written record on the earliest use of autologous tissue for restorative purposes was provided by Sushruta, an Indian professor who used a rotational flap to reconstruct mutilated nose (Bhishagratna, 1916). Fast forward to the 21st century, the tissue engineering field has witnessed significant advancements where more established researches using three dimensional (3D) engineered tissues to regenerate new tissues such as cornea (Shimmura et al., 2003; Tsai, Li, & Chen, 2000), heart valve (Flanagan