



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

BY

ROZLIN BINTI ABDUL RAHMAN

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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 مازقاً كبيراً للعظام. وكونه هيكلاً بسيطاً، مع عدم وجود النسيج العصبي وعدم ارتباطه بالأوعية الدموية فالغضروف المفصلي لديها القليل من القدرة على إصلاح نفسه عند التلف. 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) و Dulbecco 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 بـ BMSCs 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 تتقف لمدة ثلاثة أسابيع وبعد ذلك تم زرعها في نموذج أرنب. وقد تم في المختبر تقييم غضروفي المبنى بالتفتيش الإجمالي، تكاثر الخلايا الفحص، التعبير الجيني، إنتاج غلكسمينوغلكن المكبرت (sGAG) والأنسجة في الأسبوع 1 و 2 و 3 في حين تم حصادها في الجسم الحي بيني 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 كل من المختبر والمجراة. واقترحت هذه الدراسة إلى أن PLGA / الليفين عند التصنيف مع BMSCs المحفزة التي خضعت للتلاعب الأمل 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 أن 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 وتم تطبيقها ضمن الحدود المسموح بها التي حددها الشريعة الإسلامية.

APPROVAL PAGE

The thesis of Rozlin Binti Abdul Rahman has been approved by the following:

Munirah Sha'ban
Supervisor

Ahmad Hafiz Zulkifly
Co-Supervisor

Aminudin Che Ahmad
Co-Supervisor

Abdurezak Abdulahi Hashi
Co-Supervisor

Solachuddin Jauhari Arief
Internal Examiner

Norzana Abdul Ghafar
External Examiner

Ismail Zainol
External Examiner

Norlelawati A. Talib
Chairman

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.*

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TABLE OF CONTENTS

Abstract.....	ii
Abstract in Arabic.....	iii
Approval Page.....	iv
Declaration.....	v
Copyright Page.....	vi
Dedication.....	vi
Acknowledgements.....	viii
Table of Contents.....	ix
List of Tables.....	xiv
List of Figures.....	xv
List of Abbreviations.....	xvii
List of Symbols.....	xxi

CHAPTER ONE: INTRODUCTION.....	1
1.1 Background of the Study.....	1
1.2 Rationale of the study.....	8
1.3 Objective.....	9
1.3.1 General Objective.....	9
1.3.2 Specific Objectives.....	9
1.4 Hypotheses.....	10

CHAPTER TWO: LITERATURE REVIEW.....	11
2.1 Structure and Composition of Articular Cartilage.....	11
2.1.1 Water.....	11
2.1.2 Collagen and Chondrocytes.....	12
2.1.3 Proteoglycans.....	13
2.1.4 Types of Cartilage.....	14
2.1.5 Articular Cartilage Zones.....	15
2.2 Pathological Conditions Related to Articular Cartilage.....	16
2.3 Treatments for Articular Cartilage Defect.....	17
2.3.1 Osteochondral Autograft Transplantation Surgery (OATS) and Mosaicplasty.....	18
2.3.2 Microfracture.....	19
2.3.3 Autologous Chondrocytes Implantation (ACI).....	20
2.4 Tissue Engineering.....	22
2.4.1 Cell Sourcing.....	23
2.4.1.1 Chondrocytes.....	23
2.4.1.2 Stem cells.....	24
2.4.1.3 Embryonic stem cells (ESCs).....	25
2.4.1.4 Mesenchymal stem cells (MSCs).....	25
2.4.2 Biomaterial Scaffolds.....	27
2.4.2.1 Natural scaffolds.....	28
2.4.2.2 Synthetic scaffolds.....	31
2.4.3 Signalling Molecules.....	33
2.5 Bioethical Issues in Tissue Engineering from the Islamic Perspective ..	35

CHAPTER THREE: METHODOLOGY	38
3.1 Animal Model.....	38
3.1.1 Ethical Approval.....	38
3.2 Harvesting of Bone Marrow Mesenchymal Stem Cells (BMSCs).....	38
3.3 Monolayer Cell Culture.....	39
3.3.1 Group 1: The Iliac Crest and the Femoral BMSCs Cultured in FD.....	40
3.3.2 Group 2: The Iliac Crest and the Femoral BMSCs Cultured in α -MEM.....	40
3.4 Growth Kinetic Profile Analysis.....	42
3.5 Preparation of Fresh Plasma-Derived Fibrin.....	43
3.6 Fabrication of Three Dimensional (3D) Microporous Poly(Lactic- Co-Glycolic Acid) Scaffolds.....	44
3.7 <i>In Vitro</i> Three Dimensional (3D) Construct Formation.....	44
3.8 Macroscopic Observation of Engineered Construct.....	45
3.8.1 Gross Appearance of the <i>In Vitro</i> Construct.....	45
3.8.2 Simple Palpation Test of the <i>In Vitro</i> Construct using Forceps by Blinded Assessor.....	45
3.8.3 Gross Morphology Assessment of <i>In Vivo</i> Autologous Construct in Rabbit Model.....	46
3.9 Cell Proliferation Assay.....	48
3.10 Histology and Immunohistochemistry Evaluation.....	49
3.10.1 Haematoxylin and Eosin (H&E) Staining.....	49
3.10.2 Alcian Blue Staining.....	50
3.10.3 Safranin O Staining.....	50
3.10.4 Immunohistochemistry Staining.....	50
3.10.5 Modified O’Driscoll Scoring for <i>In Vivo</i> Autologous Construct in Rabbit Model.....	51
3.11 Two-Step Reverse Transcription Polymerase Chain Reaction (PCR).....	54
3.11.1 RNA Isolation.....	54
3.11.2 Complementary DNA (cDNA) Synthesis.....	54
3.11.3 Polymerase Chain Reaction (PCR) and Gel Electrophoresis.....	55
3.12 Sulphated Glycosaminoglycan (sGAG) Production Assay.....	56
3.13 Biomechanical Assessment of the <i>In Vivo</i> Autologous Construct in Rabbit Model.....	57
3.14 <i>In vivo</i> Autologous Implantation in Rabbit Model.....	58
3.14.1 Pre-Operation Preparation.....	58
3.14.2 Preparation of Ketamine-Tilatamine-Xylazine (KTX) Cocktail for Anaesthesia.....	59
3.14.3 Intra-Operative Procedure.....	60
3.14.4 Intra-Operative Care.....	63
3.14.5 Post-Operative Care.....	63
3.14.6 Euthanasia.....	63
3.15 Method for Exploring the Bioethical Issues on Tissue Engineering from the Islamic Perspective.....	64

CHAPTER FOUR: EVALUATION OF BONE MARROW MESENCHYMAL STEM CELLS (BMSC) GROWTH IN VITRO65

4.1 Abstract.....	65
4.2 Introduction.....	65
4.3 Materials and Methods	68
4.4 Results	68
4.4.1 Monolayer Cultured BMSCs Morphology	68
4.4.2 Growth Kinetic Analysis.....	72
4.4.2.1 Cells viability.....	72
4.4.2.2 Total cell count	74
4.4.2.3 Cells growth rate.....	76
4.4.2.4 Number of cell doubling.....	78
4.4.2.5 Population doubling time.....	80
4.5 Discussion.....	82
4.6 Conclusion	87

CHAPTER FIVE: THE POTENTIAL OF THREE DIMENSIONAL CONSTRUCT ENGINEERED FROM POLY (LACTIC-CO-GLYCOLIC ACID) PLGA/FIBRIN SEEDDED WITH RABBIT MESENCHYMAL STEM CELL FOR IN VITRO CARTILAGE TISSUE ENGINEERING88

5.1 Abstract.....	88
5.2 Introduction.....	89
5.3 Materials and Methods	92
5.4 Results	93
5.4.1 Gross Morphology of Constructs.....	93
5.4.2 Histological Evaluation of <i>In Vitro</i> Construct	96
5.4.3 Immunohistochemistry Evaluation of <i>In Vitro</i> Construct.....	98
5.4.4 Measurement of Cell Proliferation Assay of <i>In Vitro</i> Construct ..	100
5.4.5 Two-Step Reverse Transcription Polymerase Chain Reaction (RT-PCR).....	101
5.4.6 Sulphated Glycosaminoglycan (sGAG) Production Assay	103
5.5 Discussion.....	104
5.6 Conclusion	113

CHAPTER SIX: OSTEOCHONDRAL DEFECT REPAIR VIA AUTOLOGOUS IMPLANTATION OF THREE DIMENSIONAL CONSTRUCT ENGINEERED FROM POLY (LACTIC-CO-GLYCOLIC ACID) PLGA/FIBRIN HYBRID SCAFFOLD SEEDDED WITH BONE MARROW MESENCHYMAL STEM CELLS115

6.1 Abstract.....	115
6.2 Introduction.....	116
6.3 Materials and Methods	118
6.4 Results	119
6.4.1 Gross Morphology Assessment using the International Cartilage Repair Society (ICRS) Classification	119
6.4.2 Histological and Immunohistological Evaluation.....	121
6.4.3 Two-Step Reverse Transcription Polymerase Chain Reaction (RT-PCR).....	125
6.4.4 Sulphated Glycosaminoglycan (sGAG) Production Assay	127

6.4.5 Biomechanical Assessment.....	128
6.5 Discussion.....	129
6.6 Conclusion	134
CHAPTER SEVEN: BIOETHICAL ISSUES IN TISSUE ENGINEERING AND REGENERATIVE MEDICINE FROM ISLAMIC PERSPECTIVE:	
A PREAMBLE.....	135
7.1 Abstract.....	135
7.2 Introduction.....	135
7.3 <i>Maqasid Al-Shari'ah</i> as a Basis for Bioethical Framework	138
7.3.1 Protection of Religion	138
7.3.2 Protection of Life	139
7.3.3 Protection of Progeny.....	139
7.3.4 Protection of Mind	140
7.3.5 Protection of Wealth	141
7.4 Tissue Engineering and Regenerative Medicine	142
7.5 Methodology.....	144
7.6 Discussion.....	145
7.6.1 Exploring the Ethics of Tissue Engineering and Regenerative Medicine from the Islamic Perspective	145
7.6.2 The Role of Intention/ <i>Niyyat</i> in Islam.....	147
7.6.3 Cell Sourcing in Tissue Engineering	148
7.6.4 Genetic Modification of Cells in Tissue Engineering: Work in Relation to Cloning.....	153
7.6.5 Proof of Concept Research Using Animal Models.....	156
7.6.6 Tissue Engineered Medical Products (TEMPs) Implantation.....	159
7.7 Conclusion	161
CHAPTER EIGHT: CONCLUSION	163
8.1 Overview.....	163
8.1.1 Evaluation of Bone Marrow Mesenchymal Stem Cells (BMSCs) Growth <i>In Vitro</i>	164
8.1.2 Potential of Three Dimensional Construct Engineered from Poly (Lactic-Co-Glycolic Acid) PLGA/Fibrin Seeded with Rabbit Mesenchymal Stem Cells (BMSCs) for <i>In Vitro</i> Cartilage Tissue Engineering.....	164
8.1.3 Osteochondral Defect Repair via Autologous Implantation of Three Dimensional Construct Engineered from Poly (Lactic- Co-Glycolic Acid) PLGA/Fibrin Hybrid Scaffold Seeded with Rabbit Bone Marrow Mesenchymal Stem Cells (BMSCs).....	165
8.1.4 Bioethical Issues in Tissue Engineering and Regenerative Medicine from Islamic Perspective: A Preamble	165
8.2 Future Direction.....	166
REFERENCES.....	169

APPENDIX I	: ETHICAL APPROVAL	202
APPENDIX II	: GRANT APPROVAL (ERGS 11-005-0005).....	203
APPENDIX III	: FULL ARTICLES / PROCEEDINGS / ABSTRACTS CONTRIBUTED TO THIS THESIS	204
APPENDIX IV	: LIST OF OTHER ARTICLES / PROCEEDINGS / ABSTRACTS.....	221
APPENDIX V	: AWARD	222

LIST OF TABLES

<u>Table No.</u>		<u>Page No.</u>
3.1	International Cartilage Repair Society (ICRS) Macroscopic Evaluation of Cartilage Repair	47
3.2	Modified O’Driscoll Histological Cartilage Repair Score	53
3.3	Primers Used in the RT-PCR Analysis. F: Forward (Sense) Primer; R: Reverse (Anti-Sense) Primer	56
3.4	Anaesthesia Regime for the Combination of Ketamine, Tilatamine/Zolazepam and Xylazine	59
4.1	The Comparison for Cell Viability (%) Throughout the Passages Between Different Samples of BMSCs	73
4.2	The Comparison for Total Cell Count Throughout the Passages Among Different Samples of BMSCs	75
4.3	The Comparison for Growth Rate (cells/day/cm ²) Throughout the Passages in Different Samples of BMSCs	77
4.4	The Comparison for Number of Cell Doubling Throughout the Passages Among Different Samples of BMSCs	79
4.5	The Comparison for Population Doubling Time Throughout the Passages Among Different Samples of BMSCs	81
6.1	Scores According to International Cartilage Repair Society Classification	121
6.2	Scores According to the Modified O’Driscoll Histological Grading Scale	122

LIST OF FIGURES

<u>Figure No.</u>		<u>Page No.</u>
3.1	(A) The Operation Theatre, (B) The Anaesthetized Rabbit on the Operating Table, Ready for Surgery.	58
3.2	Intraoperative Procedure: (A) The Surgical Site Was Cleaned with Povidone Iodine (B) Draping with Sterile Surgical Draping Cloth (C) Locating the Intended Incision Site (D) Skin Incision Done (E) Opening the Fascia and Muscle (F) Knee Joint Exposed to Visualize the Medial Femoral Condyle.	61
3.3	Intraoperative Procedure: (G) Drilling with Drill Bit (H) Full Thickness Osteochondral Defect on the Medial Femoral Condyle (I) Inserting PLGA/Fibrin Construct (J) Press Fitting the PLGA/Fibrin Construct into the Defect (Note: Similar Procedure Was Done on the Lateral Femoral Condyle to Implant the PLGA Construct) (K) Wound Closing (L) Wound Dressing	62
4.1	Day 8 of Passage 0 (P0) Monolayer Cells Culture. Morphological Appearance of BMSCs Aspirated from Iliac Crest (IC) and Femur (F) Regions Cultured in FD and α -MEM. Cells are Relatively Small in Size and Showed a Constant Mixture of Cellular Appearance that Varies from Polygonal to More Spindle-Shaped and Elongated Morphology.	70
4.2	Day 5 of Passage 3 (P3) Monolayer Cells Culture. Morphological Appearance of BMSCs Aspirated from Iliac Crest (IC) and Femur (F) Regions Cultured in FD and α -MEM. Most Cells Exhibited the Fibroblastic Morphology	71
5.1	The Dimension of PLGA Scaffolds A) Diameter: 7mm and B) Thickness: 3 mm Measured using a Vernier Calliper.	94
5.2	An Empty PLGA Scaffold	94
5.3	SEM Image of the Empty PLGA Scaffold	94
5.4	Gross Morphology of <i>in Vitro</i> Constructs. Both PLGA/Fibrin and PLGA Constructs Showed Almost Similar Morphological Appearance at Week 1 of <i>in Vitro</i> Culture (Figure 5.4 A). At Week 2, PLGA/Fibrin Construct Demonstrated Slightly Smoother Surface When Compared	

	to PLGA Construct (Figure 5.4 B). At Week 3, the <i>in Vitro</i> PLGA/Fibrin Construct Appeared to be Whiter, Glossier and Smoother Than PLGA Construct (Figure 5.4 C). Both Constructs have a Slight Reduction in Size Throughout the Culture Period.	95
5.5	Histological Observations of PLGA/Fibrin and PLGA Constructs <i>in Vitro</i> via H&E Staining (Magnification of 10x and Scale Bar: 100µm).	97
5.6	Histological Observations of PLGA/Fibrin and PLGA Constructs <i>in Vitro</i> via Alcian Blue Staining (Magnification of 10x and Scale Bar: 100µm).	97
5.7	Histological Observations Of PLGA/Fibrin and PLGA Constructs <i>In Vitro</i> Via Safranin-O Staining (Magnification of 10x and Scale Bar: 100µm).	98
5.8	Immunohistochemical Observations of PLGA/Fibrin and PLGA Constructs <i>in Vitro</i> . The Sections were Stained with <i>COL2A1</i> (Figure 5.6 A-F) and <i>COL1A1</i> (Figure 5.6 G-L) Staining (Magnification of 10x and Scale Bar: 100µm).	99
5.9	Proliferation of BMSCs Seeded on PLGA/Fibrin and PLGA Scaffolds During the Cultivation Time of 21 Days of <i>in Vitro</i> Culture. PLGA/Fibrin Construct Showed Significantly Higher Cell Proliferation on Day-7, Day-14 and Day-21 Compared to PLGA Construct (P<0.05).	100
5.10	RT-PCR Analysis of the mRNA Expression of <i>COL2A1</i> , <i>ACAN</i> , <i>SOX9</i> , <i>COL9A1</i> and <i>COL1A1</i> in the <i>in Vitro</i> PLGA/Fibrin and PLGA Constructs at 1, 2 and 3 Weeks Of Culture.	102
5.11	The Relative Sulphated Glycosaminoglycan (sGAG) Contents (%) after 1, 2 and 3 Weeks of <i>in Vitro</i> Culture. The Symbol “*” Indicates a Statistical Significance (P<0.05).	103
6.1	Macroscopic Observation of the Repaired Defects in the Three Groups at Week 6 (A-C) and Week 12 (D-F) After Implantation. Arrows Depicted the Repaired Defect Area.	120
6.2	H&E, Alcian Blue and Safranin O Staining of Sections from PLGA and PLGA/Fibrin Group at 6 Weeks Post Implantation. Arrow Demonstrated Some Defects or Fissure in the Regenerated Tissue (Magnification of 100x and Scale Bar: 100µm).	123

6.3	H&E, Alcian Blue and Safranin O Staining of Sections from PLGA and PLGA/Fibrin Group at 12 Weeks Post Implantation (Magnification Of 100x and Scale Bar: 100µm).	123
6.4	<i>COL2A1</i> Immunohistological Staining of Sections in the PLGA and PLGA/Fibrin Group at 6 Weeks and 12 Weeks After Implantation (Magnification Of 100x and Scale Bar: 100µm).	124
6.5	RT-PCR Analysis of the mRNA Expression of <i>COL2A1</i> , <i>ACAN</i> , <i>SOX9</i> , <i>COL9A1</i> and <i>COL1A1</i> in the PLGA/Fibrin and PLGA Constructs at 6 Weeks and 12 Weeks After Implantation.	126
6.6	The Relative sGAG Production at 6 Weeks and 12 Weeks Post Implantation. PLGA/Fibrin Group Showed Significantly Higher Relative sGAG Content as Compared to PLGA Group (P<0.05).	127
6.7	Compression Test at 12 Weeks Post Implantation with the PLGA/Fibrin Group Shows Significantly Higher Mechanical Strength Compared to the PLGA Group (P<0.05).	128
7.1	Visualizing TERM, from Bench to Bed-Side. The Gap Between Bench and Bed Exists Until Now.	147

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 ₂ Cl 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 ₂ O 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 ₂ HPO 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 (Bhishagrata, 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