SCHOOL OF MATERIALS AND MINERAL RESOURCES ENGINEERING
UNIVERSITI SAINS MALAYSIA

EFFECT OF LEACHING AGENTS ON POLYURETHANE COMPOSITE SCAFFOLDS CONTAINING BIOACTIVE GLASS

By

LEE ANGIE

Supervisor: Dr. Syazana Ahmad Zubir

Dissertation submitted in partial fulfillment
of the requirements for the degree of Bachelor of Engineering with Honours
(Materials Engineering)

Universiti Sains Malaysia

JUNE 2017

DECLARATION

I hereby declare that I have conducted, completed the research work and written the dissertation entitled "**Effect of Leaching Agents on Polyurethane Composite Scaffolds Containing Bioactive Glass**". I also declare that it has not been previously submitted for the award of any degree or diploma or other similar title of this for any other examining body or University.

Name of student : Lee Angie Signature:

Date :

Witnessed by:

Supervisor : Dr. Syazana Ahmad Zubir Signature:

Date :

ACKNOWLEDGEMENTS

This research was supported by Dr. Syazana Ahmad Zubir, the supervisor of the project. I wish to express my sincere thanks to Dr. Syazana Ahmad Zubir for her assistance with the techniques used and methodology that greatly facilitated the research process. I am extremely thankful and indebted to her for sharing expertise, and her sincere and valuable guidance and her encouragement that was extended to me. Besides that, I would also wish to express my gratitude to Dr. Tuti, to whom I seek advice from when Dr. Syazana was on her maternity leave.

I would like to take this opportunity to express my gratitude to all of the lecturers, laboratory and workshop (*bengkel*) technicians for their help and support for this research throughout this period. I also thank my parents and family members as well as my fellow friends and coursemates for their constant encouragement, support and attention. Moreover, I would like to express my gratitude to the postgraduate students who had provided insights and expertise that has greatly assisted the research.

I also place in record, my sense of gratitude to one and all, who directly or indirectly, have lent their hand in this venture.

TABLE OF CONTENTS

Contents	Page
DECLARATION	ii
ACKNOWLEDGEMENTS	iii
TABLE OF CONTENTS	iv
LIST OF TABLES	viii
LIST OF FIGURES	ix
LIST OF ABBREVATIONS	xi
LIST OF CHEMICAL FORMULAE	xii
LIST OF SYMBOLS	xiii
ABSTRAK	xiv
ABSTRACT	xvi
CHAPTER 1 INTRODUCTION	1
1.1 Research Background	1
1.2 Problem Statement	3
1.3 Research Objectives	5
1.4 Scope of Research / Thesis Outline	5
CHAPTER 2 LITERATURE REVIEW	8
2.1 Introduction to Bone Grafting	8
2.2 Alloplastic Bone Graft	10
2.3 Characteristics of Synthetic Bone Graft	11
2.4 Bone Graft Materials	13
2.4.1 Polyurethane for Polymer Matrix Composite Scaffold	14
2.4.2 Bioactive Glass as Reinforcement in Polymer Matrix Composite Scaffold	18
2.5 Scaffold Fabrication Techniques	21
2.5.1 Conventional Techniques	21
2.5.2 Advanced Techniques	24

2.6 Salt-Particle Leaching 28
2.6.1 Advantages 29
2.6.2 Disadvantages 30
2.6.3 Applications 30
2.7 Leaching Agents 31

CHAPTER 3 EXPERIMENTAL PROCEDURE 35
3.1 Raw Materials 35
3.2 Fabrication and Synthesis 37
3.2.1 Alumina Mould 37
3.2.2 Alumina Crucible 37
3.2.3 45S5 Bioactive Glass 40
3.2.4 PU-BG Scaffold 43
3.3 Characterisation and Testing 45
3.3.1 Particle Salt Analysis (PSA) 45
3.3.2 Optical Microscopy (OM) 45
3.3.3 X-Ray Power Diffraction (XRD) 46
3.3.4 X-Ray Fluorescence (XRF) Spectrometry 46
3.3.5 Fourier Transform Infrared Spectroscopy (FTIR) 46
3.3.6 Thermal Gravimetric Analysis (TGA) 47
3.3.7 Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy (EDX) 48
3.3.8 Compression Testing 48
3.3.9 Total Porosity 49

CHAPTER 4 RESULTS AND DISCUSSION 50
4.1 Introduction 50
4.2 Polyurethane (PU) 51
4.2.1 Fourier Transform Infrared Spectroscopy (FTIR) analysis 51
4.2.2 Thermogravimetric Analysis (TGA) and Derivative Thermo-Gravimetric (DTG) analysis 53
4.3 45S5 Bioactive Glass (BG) 55
4.3.1 X-ray Diffraction (XRD) analysis 55
4.3.2 X-rays Fluorescence (XRF) analysis 56
4.3.3 Energy Dispersive X-Ray Spectroscopy (EDX) analysis 58
4.3.4 Fourier Transform Infrared Spectroscopy (FTIR) analysis 60
4.3.5 Particle Size Analysis (PSA) 61
4.4 Leaching Agent: NaCl 63
4.4.1 Optical Microscopy and TM3000 Scanning Electron Microscope 63
4.5 Leaching Agent: $NaHCO_3$ 65
4.5.1 Particle Size Analyser and TM3000 Scanning Electron Microscope 65
4.5.2 Thermogravimetric Analysis (TGA) and Derivative Thermo-Gravimetric (DTG) analysis 67
4.6 45S5 Bioactive Glass Reinforced (PU-BG) Scaffold 69
4.6.1 Fourier Transform Infrared Spectroscopy (FTIR) analysis 69
4.6.2 Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDX) analysis 71
4.6.3 Percent Porosity 76
4.6.4 Thermogravimetric Analysis (TGA) and Derivative Thermo-Gravimetric (DTG) analysis 77
4.6.5 Compression Testing 80

CHAPTER 5 CONCLUSION AND RECOMMENDATIONS 86
5.1 Conclusion 86
5.2 Recommendations for Future Works 87

REFERENCES 89

APPENDICES 98
APPENDIX A 99
APPENDIX B 101
APPENDIX C 102
APPENDIX D 103
APPENDIX E 105

LIST OF TABLES

	Page
Table 2.1: Ideal bone graft characteristics (Moore *et al.*, 2001)	11
Table 2.2: Compositions of various bioactive glasses (Rahaman *et al*., 2011)	20
Table 2.3: Percentage composition of leaching agents	32
Table 3.1: Raw materials used and its brief description	36
Table 3.2: Amount of raw materials needed to synthesize 45S5 bioactive glass	41
Table 3.3: Different ratios of leaching agents in percentage (%)	44
Table 3.4: Different ratios of leaching agents in mass (g)	44
Table 3.5: Characterisation methods for the raw materials and composite	45
Table 4.1: Theoretical weight and experimental weight obtained for BG samples	56
Table 4.2: Elemental composition of BG sample	59
Table 4.3: Theoretical weight (Jones, 2013) and experimental weight obtained for BG	59
Table 4.4: Particle size distribution of BG powder	62
Table 4.5: Particle size distribution of $NaHCO_3$	66
Table 4.6: The percentage of porosity for porous scaffolds prepared	76
Table 4.7: Final weight of samples (residue) remained	78
Table 4.8: Relationship between bulk density, compressive strength (at 50% strain) and compressive modulus (at 50% compressive strain)	81

LIST OF FIGURES

Page

Figure 2.1: Cubic and spherical salt particle and how it influence of particle shape on the neck characteristics 34

Figure 3.1: First firing profile of crucible (biscuit firing) 39

Figure 3.2: Second firing profile of crucible 40

Figure 3.3: Heating and soaking profile of 45S5 bioactive glass 41

Figure 4.1: FTIR spectrum of commercialized PU 51

Figure 4.2: TGA and DTG thermogram of polyurethane 53

Figure 4.3: XRD pattern of the 45S5 BG powder 55

Figure 4.4: Fluorescence yield of K and L electrons (Brouwer, 2010) 58

Figure 4.5: FTIR spectrum of 45S5 bioactive glass 60

Figure 4.6: Cumulative distribution of BG particle size 61

Figure 4.7: NaCl image obtained from the Image Analyser 64

Figure 4.8: Size and shape of pores created by NaCl (sample 100NaCl) 65

Figure 4.9: Size of pores created by $NaHCO_3$ by Particle Size Analyser 66

Figure 4.10: TGA and DTG thermogram of $NaHCO_3$ 68

Figure 4.11: FTIR spectra of PU, BG and PU-BG scaffold (100NaCl and 0NaCl) 69

Figure 4.12: FTIR spectra of PU-BG scaffolds 70

Figure 4.13: Pore morphology of samples at (a) 40x and (b) 100x respectively 71

Figure 4.14: SEM images and EDX data for 100NaCl at 100x magnification 75

Figure 4.15: SEM images and EDX data for 0NaCl at 100x magnification 75

Figure 4.16: TGA and DTG thermogram of $NaHCO_3$ and scaffold samples 78

Figure 4.17: Graph of compressive stress against compressive strain for scaffold samples 81

Figure 4.18: Graph of compressive modulus at 50% strain against bulk density 82

Figure 4.19: Relationship between bulk density of scaffold samples with compressive modulus at 50% strain 83

Figure 4.20: Relationship between bulk density of scaffold samples with compressive strength at 50% strain 84

LIST OF ABBREVATIONS

BG	-	Bioactive glass
CF	-	Carbon fiber
CMC	-	Ceramics matrix composite
DTG	-	Derivative Thermo-Gravimetric
FTIR	-	Fourier Transform Infrared Spectroscopy
GF	-	Glass fiber
HA	-	Hydroxyapatite
MMC	-	Metal matrix composite
MV	-	Molecular weight
P.O.P.	-	Plaster of Paris
PCL	-	Polycaprolactone
PGA	-	Polyglycolide
PLA	-	Polylactide
PMC	-	Polymer matrix composite
PMMA	-	Poly(methyl methacrylate)
PTFE	-	Polytetrafluoroethylene
PE	-	Polyethylene
PET	-	Polyethylene terephthalate
PU	-	Polyurethane
SEM	-	Scanning Electron Microscopy
TGA	-	Thermogravimetric Analysis
THF	-	Tetrahydrofuran
UTM	-	Universal Testing Machine

LIST OF CHEMICAL FORMULAE

Al_2O_3	-	Aluminium oxide
$CaCO_3$	-	Calcium carbonate
CaO	-	Calcium oxide
Na_2CO_3	-	Sodium carbonate
NaO	-	Sodium oxide
P_2O_5	-	Phosphorus pentoxide
SiC	-	Silicon carbide
SiO_2	-	Silicon dioxide

LIST OF SYMBOLS

cm	-	centimeter
kg	-	kilogram
MPa	-	mega Pascal
μm	-	micrometer
mg	-	milligram
ml	-	milliliter
min	-	minute
mm	-	millimeter
vol	-	volume
wt. %	-	weight percent
˚C	-	degree Celsius

KESAN EGEN LARUT LESAP TERHADAP PERANCAH KOMPOSIT POLIURETANA YANG MENGANDUNGI KACA BIOAKTIF

ABSTRAK

Lapan sampel perancah poliuretana komposit berliang yang mengandungi 45S5 kaca bioaktif telah dihasilkan melalui kaedah garam larut lesap. Setiap sampel mengandungi 80% poliuretana dan 20% 45S5 kaca bioaktif. Dua ejen larut lesap yang berbeza, iaitu NaCl dan $NaHCO_3$ telah digunakan untuk menghasilkan struktur berliang untuk perancah. Nisbah antara NaCl dan $NaHCO_3$ adalah berbeza. Nisbah NaCl: $NaHCO_3$ yang digunakan ialah 100:0, 95:5, 90:10, 85:15, 80:20, 75:25, 70:30 dan 0:100. Morfologi liang bersama-sama sifat terma dan mekanikal bagi perancah berliang telah dicirikan. Ikatan yang hadir dalam perancah yang dikaji telah dikaji menggunakan Analisis Intra-Merah (FTIR), manakala morfologi liang dikaji menggunakan Mikroskopi Imbasan Elektron (SEM). Perancah berliang yang dihasilkan mempunyai liang dalam julat saiz puluhan ke ratusan mikron, yang sesuai untuk kejuruteraan tisu tulang. Kelakuan terma untuk semua sampel telah disiasat menggunakan Analisis Termogravimetri (TGA). Keputusan menunjukkan bahawa baki peratusan sisa di dalam perancah pada 700°C bertambah dengan pertambahan amaun $NaHCO_3$. Sifat mekanikal bagi perancah yang dihasilkan dikaji menggunakan ujian mampatan. Modulus mampatan bagi perancah yang dihasilkan berjulat antara 0.11-2.15 MPa. Selain itu, jumlah keliangan yang wujud di dalam perancah turut dikaji. Keliangan minima iaitu 70% biasanya diperlukan bagi aplikasi biomedikal. Sementara dalam kajian ini, majoriti

sampel yang dihasilkan mempunyai keliangan yang mencukupi untuk digunakan dalam kejuruteraan tisu tulang.

EFFECT OF LEACHING AGENTS ON POLYURETHANE COMPOSITE SCAFFOLDS CONTAINING BIOACTIVE GLASS

ABSTRACT

Eight samples of porous polyurethane composite scaffolds containing 45S5 bioactive glass were fabricated by salt-particle leaching method. Each sample contains 80% polyurethane and 20% 45S5 bioactive glass. Two different leaching agents, namely NaCl and $NaHCO_3$ were used to create the porous structure of scaffolds. The ratio between NaCl and $NaHCO_3$ were varied, with NaCl:$NaHCO_3$ percentage being 100:0, 95:5, 90:10, 85:15, 80:20, 75:25, 70:30 and 0:100. The porous scaffolds were characterized based on its pore morphology as well as its thermal and mechanical properties. Bonding properties of scaffold samples were studied using Fourier Transform Infrared Spectroscopy (FTIR), whereas its pore morphology were studied using Scanning Electron Microscopy (SEM). Porous scaffolds fabricated exhibits pores of which their sizes range from tens of microns to hundreds, which are suitable for bone tissue engineering. Thermal behaviour of samples were investigated using Thermogravimetric Analysis (TGA). Result shows that the percentage of residues remained in the scaffold (at 700°C) increases with increasing amount of $NaHCO_3$. Mechanical properties of the produced scaffolds were studied by though compression test. Compressive modulus of the fabricated scaffolds ranges from 0.11 to 2.15 MPa. Besides that, the total porosity existing in the scaffold was studied as well. A minimum porosity of 70% porosity is usually required for biomedical applications, whereby in this study, majority of the samples fabricated has sufficient porosity to be used for bone tissue engineering.

CHAPTER 1

INTRODUCTION

1.1 Research Background

In some cases where bones are broken, a temporary framework is needed to support bone tissue regeneration. The use of bone grafts is the standard to treat skeletal fractures, or to replace and regenerate lost bone, as demonstrated by the large number of bone graft procedures performed worldwide (Polo-Corrales *et al.*, 2014). Many years back, bone transplant is done based on three categories; autograft transplant, allograft transplant and xenograft transplant. Each of these methods have its own advantages and disadvantages. One very main reason where these three methods share a common limitation is that they are dependent on the availability of donors. To overcome this limitation, the focus has switched to another transplant system known as the alloplastic graft transplant, whereby bioactive synthetic materials is used as one of the components to produce a temporary scaffold (Ramakrishna *et al.*, 2016). The study of artificial scaffolds have increased over the years.

The production of a scaffold is heavily dependent on the requirements of human body. Some of the vital requirements include (1) being biocompatible and bioactive (to promote osteogenic cell attachment and osteogenesis), (2) having interconnected porous structure that can allow fluid flow, cell migration, bone ingrowth and vascularization, and (3) being able to be cut to shape so that it can fit the defect (Jones, 2013).

Artificial scaffolds of different types have been studied and produced based on bone tissue engineering requirements. Three main categories of bone graft (scaffolds) include the

(1) ceramic matrix composite (CMC), or ceramic-based scaffolds; (2) metal matrix composite (MMC), or metal-based scaffolds; and finally the (1) polymer matrix composite (PMC), or polymer-based scaffolds. Each group of materials have its own superiority. For instance, bioceramics generally show better tissue responses as compared to metals and polymers. Examples of bioceramics includes hydroxyapatite (HA) and alumina (Baino *et al.*, 2015). On the other hand, bone graft made from metals are able to sustain compressive strength and fatigue resistance. Some examples are titanium, magnesium and tantalum (Bose *et al.*, 2012; Ramakrishna *et al.*, 2001). Polymer scaffolds generally comes with a range of combinations, but most of the combinations comes with bioceramics being the reinforcement in the composite structure. These polymers used as bone grafts are generally bioactive and biodegradable at the same time, besides having high flexibility in its processing. Some examples of the polymer matrix include the PMMA, PE, PTFE and PU (Ramakrishna *et al.*, 2001).

Studies on suitable materials for scaffold production turned to polymer-bioglass composite scaffolds as this combination satisfies the requirement needed for soft tissue engineering scaffolds (Fabbri *et al.*, 2010). Thermoplastic polymers, such as polyurethane have been proposed to be used as the binding matrix of a polymer-bioglass scaffold system (Zeimaran *et al.*, 2015). However, the concept of using bioceramics as a reinforcing phase in polymeric composites is introduced due the reason that polymers suffer from insufficient strength and poor bioactivity whereas bioactive glasses suffer from low fracture toughness, brittleness and low flexibility when used alone (Zeimaran *et al.*, 2015). 45S5 bioactive glass is commonly used in the fabrication of synthetic scaffold due to its strong bond formation with bone that are so strong that it could not be removed without breaking the bone itself,

besides having better biological properties than other phosphate- and borate-based bioactive glass (Jones, 2013).

Fabrication of artificial scaffolds consisting of polyurethane and 45S5 bioactive glass may be fabricated in various conventional and advanced methods. One of the simplest conventional techniques is the solvent casting/particle leaching technique (Janik and Marzec, 2015; Loh and Choong, 2013), whereby this method is believed to be able to control the pore structure of the scaffold when the amount and size of leaching agents are altered respectively (Ryszkowska *et al.*, 2010).

NaCl has historically been one of the major choice of leaching agents used in salt-particle leaching process (Cannillo *et al.*, 2010; Ryszkowska *et al.*, 2010; Mikos and Temenoff, 2000). In this study, a mixture of NaCl-$NaHCO_3$ is studied to investigate the effect of adding in a second type of leaching agent. A mixture of NaCl-$NaHCO_3$ is said to be able to form a porous structure with homogeneously distributed pores (Zeimaran *et al.*, 2015). In order to determine the best combination of leaching agents ratio suitable for fabrication of polyurethane-45S5 bioactive glass scaffolds, different ratios of NaCl and $NaHCO_3$ are varied in this study.

1.2 Problem Statement

Bone grafting is an essential technique for internal fixation treatment that is generally used for bone fracture. Three dimensional porous scaffolds ought to possess criteria such as biocompatibility and biodegradability, besides being able to contribute mechanical support, physical, and biochemical stimuli for optimal cell growth (Loh and Choong, 2013).

In bone grafting techniques, pore structures such as porosity and pore size contribute to functionality of artificial scaffolds during biomedical applications (Loh and Choong, 2013). There are several methods to produce the porous structure in the scaffold, such as gas forming, phase separation, freeze-drying, *et cetera*. In this study, the salt-particle leaching method is used to create the pores for a scaffold.

Open, interconnected porous networks are vital for cell nutrition, proliferation, and migration for tissue vascularization and formation of new tissues (Loh and Choong, 2013). However, NaCl which has a cubic structure, is expected to have lower interconnectivities between pores due to its sharp edges at the cubic corners (Murphy *et al.*, 2002). In this study, a different salt, $NaHCO_3$ with a different shape is introduced into the system to improve the interconnectivity among the pores.

Different ratios of NaCl:$NaHCO_3$ have previously been studied. For instance, Cannillo *et al.* (2010) produced porous structure in his scaffolds using both NaCl and $NaHCO_3$. Polycaprolactone has been used as the matrix where and 45S5 bioactive glass were used as the reinforcement. The composite scaffolds were produced by means of the salt-leaching technique (Cannillo *et al.*, 2010). From the study, a combination of NaCl–$NaHCO_3$ mixture was preferable, since the sample exhibited abundant and well-developed porosity, as compared to using 100% NaCl and 100% $NaHCO_3$.

In this study, different ratios of NaCl:$NaHCO_3$ is studied. Pore sizes and shapes, as well as its homogeneity and total porosity is studied. The pore structures will then be correlated to its mechanical properties, on which the samples will be tested on the compressive strength and modulus.

1.3 Research Objectives

The research objectives of this study are:

1) To prepare a composite scaffold made of polyurethane and 45S5 bioactive glass using salt-particle leaching method.
2) To examine the effect of different ratios of leaching agents ($NaCl:NaHCO_3$) on the pore morphology and mechanical properties of a polyurethane composite scaffolds containing 45S5 bioactive glass.

1.4 Scope of Research / Thesis Outline

The main prospects of this study is to fabricate a composite scaffold made of polyurethane and 45S5 bioactive glass using salt-particle leaching method. The objective of this study is to examine the effect of different ratios of leaching agents ($NaCl:NaHCO_3$) on the pore morphology and mechanical properties of a polyurethane composite scaffolds containing 45S5 bioactive glass that is to be used in tissue engineering in the biomedical field.

In the bone tissue engineering field, the research on scaffolding materials is considered concentrated. Many materials and fabrication methods have been studied by numerous researchers.

Pore structure plays an important role in facilitating bone growth as well as allowing adequate diffusion of nutrients during tissue culture and provides sufficient surface area for cell–biomaterial interactions (Polo-Corrales *et al.*, 2014). A few parameters of the pore structure can be manipulated, namely by varying the:

(1) shape (Janik and Marzec, 2015) and sizes of the leaching particles (Shin *et al.*, 2014) which affects the shape and size of pores formed in the scaffold

(2) amount of salt particles added (Shin *et al.*, 2014) which will affect the porosity structure of the scaffold

(3) initial concentration of the polymer melt which influences the direct contacts between the leaching particles (Janik and Marzec, 2015).

Two leaching agents (NaCl and $NaHCO_3$) were used to form the pores in the composite scaffold. The ratios between the pores were manipulated, with the total amount of leaching agents remaining constant. A total of 50 g leaching agents were used for 10 wt. % of polyurethane pellets per 100 ml solvent (tetrahydrofuran) to fabricate a scaffold with 80% polyurethane and 20% 45S5 bioactive glass.

Besides understanding and studying the pore structure formed by leaching agents, the pore structure formed are prone to influence the mechanical properties of the scaffold. The fabricated scaffold is expected to be able to share mechanical load with the host bone and maintain an appropriate level of mechanical properties during degradation and remodeling (Jones, 2013). It is expected that the pore size and porosity of the scaffold will affect the mechanical properties of the scaffold (Janik and Marzec, 2015; Zeimaran *et al.*, 2015).

There were generally a few particular characterization methods and analyses performed on the fabricated scaffold samples in order to study the samples' behavior. Pore microstructure and morphology were studied using Scanning Electron Microscopy (SEM). As for the extent of bond characteristics of the samples, Fourier Transform Infrared Spectroscopy (FTIR) is used to study the extent of bonding groups' intensity present in the scaffolds. Thermal behavior of samples were studied using Thermogravimetric Analysis

(TGA). Gravimetric technique is used to study the total porosity (percentage of porosity) of scaffold samples. Mechanical properties (compressive strength) of the samples, on the other hand, were studied using the Universal Testing Machine (UTM).

CHAPTER 2

LITERATURE REVIEW

Bone grafting technology have been around for centuries. A few different concepts have emerged since the first graft surgery, leading to different discoveries, implications and improvements of the technology. The number of researches in this field continues to grow even to this date, and this research paper is based thoroughly on these concepts.

2.1 Introduction to Bone Grafting

Human bones have one very distinguishable advantage, which is the fact that it has the potential of self-regeneration after an injury. However, this healing property may be impeded beyond a certain critical size (Poh *et al.*, 2014). In the body of an injured person, bone grafting has been carried out since the 1660s. An anthropologist A. Jagharlan at Erivan Medical Centre had examined a prehistoric skull from the ancient Centre of Ishtkun and found a piece of animal bone filling in a 7 mm defect with bony regrowth around the grafted bone (Berry and Lieberman, 2012). It has been suggested that the ancient Egyptians and Greeks had once before attempted bone transplantation. In the modern age, the first documented bone graft was successfully performed in 1668 by a Dutch surgeon named Job Van Meekeren, who successfully inserted a fragment of a dog bone into the skull of an injured soldier (Berry and Lieberman, 2012). This process is known as xenograft bone grafting whereby the bone is derived from another species (usually non-living), which needs to be processed at a very high temperature to avoid contamination and immune rejection. On the

other hand, the first successfully documented autograft transplant was performed in Germany in 1821 by Philips von Walter, when he experimentally created animal bone defects (Berry and Lieberman, 2012), whereby the bone is derived from the original living host. As for what is known as the allograft bone transplant, the first documented transplant was performed in 1879 by Sir William MacEwen in Scotland, who replaced the infected proximal two thirds of a humerus in a 4-year-old boy with the tibia from another child with rickets (Berry and Lieberman, 2012).

Xenogenic bone transfer have one main advantage whereby it does not require a second procedure to harvest the host's own bone. However, since the bone is taken from other species (for example, cows), bone tissue may have a shorter lifespan which is different from human beings, which may cause the tissue to have shorter lifespan for the host.

Bone transfer by means of autograft offer no immunological rejection, have excellent success rate and have low risk of disease transmission (Zimmermann and Moghaddam, 2011). Bones transplant by autograft are able to fulfill all the four 'ideal' characteristics of bone transfer (Please refer to Table 2.1), but they do, however, have their own limitations as well. Autograft bone transplant is limited to resorption, limited availability and viability (two hours when kept in saline) for bones, morbidity potential at the donor site, increased blood loss, prolonged anaesthetic time as well as having risks of wound infection (Zimmermann and Moghaddam, 2011). Two surgeries needed to be performed on the patient, as compared to xenograft and allograft which required only one (first was to extract a healthy bone tissue, second one functions to transfer the bone to the injury site).

In the case of allograft bone transplant, the major advantages of allograft bone harvested from cadavers are its ready availability in various shapes and sizes, avoidance of

the need to sacrifice host structures, and no problems of donor-site morbidity. On the other hand, osteogenic properties (Refer to Table 2.1 for definition) of allograft bone transplant is very much lacking because of the absence of viable cells as well as having a high risk of transmission of infectious agents which is a major concern.

Tissue procurement is complex, expensive and requires additional surgery. As an alternative, attention has been focused on the use of synthetic material that holds the ability to repair or restore the functions of a defective system into a normal healthy system upon implantation, which is termed alloplastic graft.

2.2 Alloplastic Bone Graft

Alloplastic bone grafting is a method of surgically inserting synthetic materials into a human body, whereby alloplastic materials are biological materials that are manufactured in a completely synthetically manner (Ramakrishna *et al.*, 2016). The synthetic material, or alloplast, used for this purpose is called biomaterial. Biomaterials may come naturally or derived from a man-made origin that are used to direct, supplement or replace the functions of human tissues in the human body. The biomaterial is used either as such or to manufacture implantable devices or prostheses to assist in healing of bone tissue and thus to improve the quality of life of the patients. According to a report published in 1995 in London, the world market for biomaterials in the industry is estimated to be around $12 billion per year (Ramakrishna *et al.*, 2016). This illustrates the importance and demands of biomaterials in the society decades back. The use of these implantable scaffolds in tissue engineering offers the possibility for a person with failing or malfunctioning organs to create and regrow

completely natural tissues (Lu and Mikos, 1996). Studies on scaffolds have increased over the years, with more precise information of suitable scaffold characteristics for application and installment in human body.

2.3 Characteristics of Synthetic Bone Graft

As per mentioned in subtopic 2.1, application and installment of scaffolds in human body depends on certain obligatory characteristics. An ideal synthetic bone graft is a porous material that can act as a temporary framework for growth of bone tissue in 3-dimensions. According to Moore *et al.* (2001), the four characteristics that an ideal bone graft should exhibit is discussed in Table 2.1.

Table 2.1: Ideal bone graft characteristics (Moore *et al.*, 2001)

Characteristics	**Definition**
Osteointegration	The ability to bond chemically to the surface of the bone without an intervening layer of fibrous tissue
Osteoconduction	The ability to support bone growth over its surface
Osteoinduction	The ability to induce differentiation of pluripotent* stem cells from surrounding tissue to an osteoblastic** phenotype
Osteogenesis	The ability to form new bone by osteoblastic cells present within the graft material

* : stem/immature cells that can generate an entire organism and all types of cells of an adult body with a broad range of functions (Şahin *et al.*, 2016)

**: cells that are matured and responsible for formation and mineralization of bone matrix (Arnett and Henderson, 2007)

The underlying basis behind the application of bone grafts is based on the assumption that bone tissues in human body can be stimulated, if the bone graft possesses components which is able to serve as a temporary framework for growth of bone tissue (osteoconduction), whereby the components in the scaffold should contain bone forming cells (osteogenesis) and bone-inducing substances (osteoinduction).

Materials characteristics of implantable scaffolds majorly revolves around biocompatibility and biodegradability in the human system (Jones, 2013; Ramakrishna *et al.*, 2001; Lu and Mikos, 1996), and Jones (2013) proposed further some characteristics of an ideal synthetic bone graft, whereby it should:

1. be compatible and bioactive, promoting osteogenic cell attachment and osteogenesis;
2. bond to the host bone without fibrous tissue sealing it off from the body;
3. have an interconnected porous structure that can allow fluid flow, cell migration, bone ingrowth and vascularization;
4. be able to be cut to shape in theatre so that it can fit the defect;
5. degrade at a specified rate and eventually be remodelled by osteoclast action;
6. share mechanical load with the host bone and maintain an appropriate level of mechanical properties during degradation and remodelling;
7. be made by a fabrication process that can be up-scalable for mass production;
8. be sterilizable and meet regulatory requirements for clinical use.

An ideal synthetic scaffold is expected to fulfill most of the listed criteria stated (Jones, 2013). The subsequent execution is to opt for the right materials suitable for the scaffold fabrication.

2.4 Bone Graft Materials

Different types of artificial scaffolds have been studied and produced based on the requirements as per mentioned in subtopic 2.3. Three main categories of bone graft (scaffolds) include:

1. ceramic matrix composite (CMC), or ceramic-based scaffolds;
2. metal matrix composite (MMC), or metal-based scaffolds;
3. polymer matrix composite (PMC), or polymer-based scaffolds.

Combinations of CF/C, SiC/C are examples of CMC scaffolds, used especially in dental implantations (Ramakrishna *et al.*, 2001). Many bioceramics are chosen as a choice of implantation in human body, besides using it as a whole, as bioceramics generally show better tissue responses as compared to metals and polymers. Some bioceramics include the hydroxyapatite (HA) and alumina. Alumina, for instance, is a widely-used crystalline ceramic to fabricate components of knee and hip joint prostheses, such as the femur head and tibial plate). Alumina is chosen due to its high-strength for load-bearing applications, excellent wear resistance and bioinertness (Baino *et al.*, 2015). Given the mechanical rigidity and inorganic nature of bioceramics, the application of these materials usually revolves around hard tissue repair, such as bones and teeth, besides being able to regenerate various types of damaged soft tissues (Baino *et al.*, 2016; Baino *et al.*, 2015).

An example of MMC scaffold is the Titanium/PMMA (Ramakrishna *et al.*, 2001). Bone tissue need to be able to sustain compressive strength and fatigue resistance, and metals possesses this nature. Certain porous scaffolds are made out of metallic structures entirely, predominantly titanium, magnesium, and tantalum, as materials used for bone replacement.

However, there are concerns regarding metal ion release if implanted into the body (Bose *et al.*, 2012).

Classes of PMC scaffolds cover a broad range of combinations, with some of the examples being CF/PMMA, CF/PTFE, CF/PE, BG/PE, GF/PU, GF/PMMA, CF/PMMA, PET/PU and PTFE/PU (Ramakrishna *et al.*, 2001). The classes of polymers can be both bioactive and biodegradable at the same time, besides having the ability to be tailored easily and having high flexibility in its processing. Some of the polymers have compressive strength that are comparable to cortical bone, besides being able to control its degradation time based on its usage (Bose *et al.*, 2012).

Among all the appropriate combinations of morphology, proccessability and bioactivity, polymer-bioceramic composite scaffolds enjoy both the mechanical reliability of the polymer phase and the excellent bioactivity of the glassy phase, and thus polymer-bioceramic composite scaffolds are widely studied in many researches and used for transplantation in the human body (Fabbri *et al.*, 2010).

2.4.1 Polyurethane for Polymer Matrix Composite Scaffold

Polymers, as known to all, have a large variety to them. Currently, scaffolds are produced with natural and synthetic polymers. Natural polymers are used as the component for extracellular matrix that plays the role of maintaining the tissue structure as well as promoting cellular adhesion. Some examples of natural biopolymers include proteins, polysaccharides and glycosaminoglycans. Protein is a large biopolymer group inclusive of collagen and gelation (denatured form of collagen). This group is usually chosen to be made

as the extracellular matrix of the tissue as osteoblast adhesion and migration can be greatly enhanced due to the presence of its functional group. Chitosan on the other hand, which is an example of polysaccharides greatly enables interaction with cell membranes, besides having excellent biocompatibility and biodegradability, as well as having an intrinsic antibacterial nature. Hyaluronic acid, which is an example under the class of glycosaminoglycans, is one of the major components in an extracellular matrix present in connective tissues which possesses good visco-coelasticity, biocompatibility and non-immunogenicity, thus making it suitable to be used in biomedical applications for tissue engineering (Pina *et al.*, 2015). However, there were concerns raised on the use of natural polymers, with some being: (1) the natural polymer has complex structural composition, (2) immunogenicity and pathogenic transmission (Guo and Ma, 2014).

Thus, synthetic polymers seem to be more promising, since the synthesis process can be strictly controlled, which also offers the opportunity to govern and tailor the final microstructure (Fabbri *et al.*, 2010). A number of advantages of synthetic polymers as compared to natural polymers were reported. Synthetic polymers are able to be processed into a variety of porous structures by using traditional and advanced methods. These methods will be further introduced in subtopic 2.5. Synthetic polymers are well-considered and discussed in tissue engineering due to their potential ability to enable cell adhesion, migration proliferation and differentiation (Ryszkowska *et al.*, 2010). Possible toxicity, immunogenicity and infection risks are known to be lower in synthetic polymers with a constituent monomeric unit of a simpler structure (Gentile *et al.*, 2014).

There are a few frequently-used synthetic polymer for biomedical applications, with some examples being the aliphatic polyesters, polyanhydrides, and polyurethanes. Aliphatic

polyesters are frequently used in pre-designed 3D scaffolds whereby it is a stable porous material that does not dissolve or melt under *in vitro* tissue culture conditions. Some of its copolymers include polylactide (PLA), polyglycolide (PGA) and poly(ε-caprolactone) (PCL). Many studies on biomedical applications revolve around PLA as the extracellular matrix as PLA is biodegradable and more hydrophobic than PGA due to the methyl group present in its structure. PCL is also a widely-researched component for extracellular matrix for tissue engineering as it is degradable through various forms, such as by microorganism, hydrolytic, enzymatic or intracellular mechanism under psychological conditions (Guo and Ma, 2014).

Moving on polyanhydrides, these polymers have been synthesized from low-cost sources and have been manipulated to meet desirable characteristics for tissue engineering. Polyanhydrides are biocompatible and degradable under *in vivo* tissue culture conditions. However, polyanhydrides are very much designed initially for drug delivery systems as it is very hydrophobic and undergoes degradation through surface erosion. More researches on how polyanhydrides can contribute in the tissue engineering field have been explored more recently as compared to the early stages (Guo and Ma, 2014).

Polyurethanes, which remained as one of the most popular groups of biomaterials, are used for a broad range of biomedical applications. They are popular due to their segmented-block structural character, which allows them with a broad range of versatility in terms of mechanical, physical and biological properties, blood and tissue compatibility, and most importantly their biodegradability (Guo and Ma, 2014). Polyurethanes contains soft segments that are primarily composed of polyester chains, and hard segments that are mainly consisted of polyurethane blocks. Due to these segments, polyurethanes are said to have elastic properties, thermoplasticity and durability. The microdomain structure of these

segments influences its main characteristics, whilst its mechanical properties and processability is greatly influenced by its structure (Ryszkowska *et al.*, 2010). Polyurethanes can be stable or degradable, or can be hydrophobic or hydrophilic, depending on how the polymer is synthesized as well as its composition, thus it is widely used as the matrix of a composite scaffold. These factors encourages the usage of polyurethane as one of the components in the scaffold (Janik and Marzec, 2015).

Fabrication of biomedical devices, such as cardiovascular catheters, blood pump diaphragms, implantable pacemakers' coating materials and other biomedical products have been manufactured using polyurethane. Due to polyurethanes being biodegradable, scaffolds for bone tissue engineering have been proposed, albeit having not much work being conducted on this matter (Ryszkowska *et al.*, 2010).

However, despite all amenities, as compared to other bioactive materials, polyurethanes are lacking of bioactive factors and cytocompatibility (Huang *et al.*, 2009). Scaffolds made from synthetic polymers do not exhibit adequate mechanical properties and bioactive behaviour which is a disadvantage for bone tissue engineering applications. Additional bioactive material is required to be included in during the processing to improve bioactivity of the scaffold, as well as to improve the mechanical properties of the structure. One of the ways suggested to improve polymer scaffolds' properties and performance for bone tissue engineering is the development of biodegradable polymer/bioactive ceramic composites.

2.4.2 Bioactive Glass as Reinforcement in Polymer Matrix Composite Scaffold

In the process of materials selection for bone tissue engineering and tissue regeneration, a crucial aspect to be considered is the ability to withstand a certain mechanical load that the structure will experience *in vivo* (Baino *et al.*, 2015). Thus, as per mentioned in the previous subtopic, the development of biodegradable polymer/bioactive ceramic composites is greatly explored. However, due to ceramics' brittle nature (low fracture toughness), its applications are rendered limited. Thus, a compromise between higher toughness and ability to withstand mechanical load is needed. As per mentioned previously, polymer which lacks of mechanical strength and bioactivity requires a foreign component to overcome its shortcomings. One suggested approach to overcome these problems is combining both polymers and bioceramics to bring out the best aspects of both materials. Typically, bioceramics are added as fillers or coatings to polymers to improve its mechanical properties, besides improving and enhancing bioactivity (Baino *et al.*, 2015).

The gist of choosing a suitable bioceramic is based on the concept whereby the chosen material is able to mimic ion composition in bones to trigger regeneration. The most commonly used ceramics are hydroxyapatite (HA), β-tricalcium phosphate (β-TCP), and biphasic calcium phosphate (BCP), which is a mixture of HA and TCP (Lee *et al.*, 2014). Due to the fact that these bioceramics have the same ions as human bone, these inorganic materials have received most attention for bone regeneration applications. As compared to β-TCP, HA resorbs slowly and undergoes minimal conversion to a bone-like material after implantation in human body. However, in terms of strength, β-TCP scaffolds of the same porosity is said to have lower strength than HA scaffolds, this restricting its use in the repair of load bearing bones. The introduction of BCP enables the use of different HA to β-TCP

ratios which allows the manipulation of the rate of degradation as well as other properties (Rahaman *et al.*, 2011). Other worth-mentioning candidates include the bioactive glasses (such as 45S5 Bioglass) and apatite-wollastonite glass-ceramics, which are also good examples of bioactive inorganic materials suitable to be used as fillers in biodegradable polymers.

According to a group of researchers (Huang *et al.*, 2009; Rezwan *et al.*, 2006; Miao *et al.*, 2004), out of all choices of bioceramics, bioactive glasses have some advantages over the others, such as possessing excellent osteoconductivity and bioactivity, controllable biodegradability and ability to induce osteogenesis and angiogenesis. Bioactive glass are divided into three categories, namely the silicate-based glasses, phosphate-based glasses and the borate-based glasses. Presently, the interest in borate glasses has increased, most likely due to very encouraging clinical results of healing of chronic wounds that would not heal under conventional treatment. It is very likely due to its fast dissolution (relatively faster compared to silicate-based glasses) that causes the response of soft tissues. Phosphate-based glasses, on the other hand, have a benefit of rapid solubility. However, even after a long research period of 40 years in bioactive glasses, silicate-based glasses still proved to have relatively good biological properties as compared to the other two classes. A very appealing example is the original 45S5 bioactive glass composition (Jones, 2013).

If the formulation of bioglass is properly designed, bioglasses can positively interact even with soft tissues. This behaviour was first observed in a family of glasses belonging to the SiO_2–Na_2O–CaO–P_2O_5 system, investigated by Hench and co-workers since 1970s (Fabbri *et al.*, 2010). Since the report of its bone-bonding properties, the bioactive glass designated 45S5 Bioactive Glass (BG), or sometimes referred to by its commercial name

Bioglass®, has been the most widely researched bioactive glass for biomedical applications (Rahaman *et al.*, 2011).

As emphasized by Fabbri *et al.* (2010), the glass termed 45S5 Bioglass is the best-known and widely investigated glass due to its extreme bioactivity. This glass is a silicate-based glass whereby its 3D glass forming SiO_2 network consists of silicon atoms are being fourfold coordinated to oxygen atoms. The key features which is responsible for its bioactivity of the bioactive glass are its (1) low SiO_2 as compared to other more chemically durable silicate-based glasses, (2) high percentage of CaO and Na_2O which functions as glass network modifiers, and (3) high CaO/P_2O_5 ratio. The composition of various bioactive glasses is shown in Table 2.2 (Rahaman *et al.*, 2011). When it is implanted in the body, the bioglass induces an interfacial bioactive behavior (good biocompatibility). Furthermore, the dissolution products of bioactive glass exert control over genetic factors of bone growth (de Oliveira *et al.*, 2012).

Table 2.2: Compositions of various bioactive glasses (Rahaman *et al.*, 2011)

Composition (wt. %)	**45S5**	**13-93**	**6P53B**	**58S**	**70S30C**	**13-93B1**	**13-93B3**	**$P_{50}C_{35}N_{15}$**
Na_2O	24.5	6.0	10.3	0	0	5.8	5.5	9.3
K_2O	0	12.0	2.8	0	0	11.7	11.1	0
MgO	0	5.0	10.2	0	0	4.9	4.6	0
CaO	24.5	20.0	18.0	32.6	28.6	19.5	18.5	19.7
SiO_2	45.0	53.0	52.7	58.2	71.4	34.4	0	0
P_2O_5	6.0	4.0	6.0	9.2	0	3.8	3.7	71.0
B_2O_3	0	0	0	0	0	19.9	56.6	0

Bioactive glass is structurally brittle, but has several desirable characteristics to capitalize upon such as (1) controllable degradation rate, (2) ionic release with osteogenic potential, (3) capacity to become HA-like material, and (4) having good bonding affinity to bone (Lee *et al.*, 2014). Thus, many processing techniques have been developed to fabricate polyurethane-45S5 bioglass composite scaffolds to meet the requirements of the application.

2.5 Scaffold Fabrication Techniques

Various techniques have been used for the fabrication of 3-dimensional (3D) scaffolds. Methods to produce porous scaffold can be divided into two groups; conventional and advanced (Janik and Marzec, 2015).

2.5.1 Conventional Techniques

Typically, conventional techniques to fabricate a 3D scaffold includes salt-particle leaching, gas forming, phase separation and freeze-drying. Conventional fabrication techniques, albeit simple, has its downsides which includes not being able to control precisely the internal structure of the scaffold, not being able to achieve complex architecture, as well as requiring good fabrication skills to maintain consistency in a scaffold's architecture (Loh and Choong, 2013).

(i) Salt-Particle Leaching

Salt-particle leaching may be conjointly known as porogen leaching. Salt crystals or porogen are first dispensed into a mould. Molten/liquid polymer is then added into the mould to fill in the remaining spaces. The polymer is subsequently hardened and the salt crystals present is removed via dissolution in a solvent such as water or alcohol. Pores will form in the hardened polymer once all of the salt crystals are leached out (Loh and Choong, 2013).

This techniques enables the size of the pores in the scaffold to be controlled by choosing the desired size of salt crystals to be placed into the mould for subsequent leaching process. The amount of salt crystals placed into the mould will then determine the percentage porosity of the scaffold (Loh and Choong, 2013).

(ii) Gas Forming

Gas forming process is somewhat similar to salt-particle leaching process, except in this technique, gas is used as the porogen instead of crystals. Polymers will first be formed into solid discs by high-temperature compression moulding, prior to the application of high-pressure carbon dioxide gas through the discs for a period of few days. The pressure is then reduced back to atmospheric level (Loh and Choong, 2013).

This technique enables the elimination of the use of harsh chemical solvents, thus dismissing the leaching step from the fabrication process. This subsequently reduces the overall fabrication time. However, through gas forming technique, pore connectivity and pore size is difficult to be manipulated. The application of high temperature during disc

formation further prohibits the use of bioactive molecules in the scaffold, which is one of the requirements of an ideal bone graft (Loh and Choong, 2013).

(iii) Phase Separation

There are different types of phase separation techniques available, namely (1) thermally-induced, (2) solid-liquid and (3) liquid-liquid phase separation. Generally, a suitable solvent will be used to first dissolve the polymer, which will subsequently be placed in a mould that encourages rapid cooling until the solvent freezes. Pores will be left behind in the polymer through freeze-drying whereby the solvent will be removed from the hardened polymer (Loh and Choong, 2013).

This technique enables the elimination of an extra leaching step, however, the addition of organic solvents such as methanol or ethanol inhibits the incorporation of bioactive molecules or cells during the scaffold fabrication. Additionally, scaffolds fabricated by phase separation technique tends to have small pore sizes, which is considered a limiting factor (Loh and Choong, 2013).

(iv) Freeze-Drying

In the freeze-drying process, polymer solutions may be used directly. There is no need to cross link monomers. Frozen water is sublimated directly into the gas phase, which results in formation of pores. Through this process, the elimination steps can be eliminated as the dispersed water molecules and polymer solvents can be removed directly from the bulk solid (Loh and Choong, 2013).

This technique enables the percentage porosity and the pore sizes of the scaffold to be controlled by the two parameters, namely the (1) water to polymer solution ratio and (2) the viscosity of the emulsion. Meanwhile, pore structure can be controlled by varying the freezing temperature of the scaffold. However, in order to increase homogeneity of scaffolds produced, this process should be controlled so as to be able to reduce heterogeneous freezing (Loh and Choong, 2013).

2.5.2 Advanced Techniques

Some of the advanced fabrication techniques include electrospinning, 3D printing and rapid prototyping. Advanced techniques were explored as conventional techniques come along with some shortcomings. For instance, scaffolds fabricated traditionally have a compressive moduli which is much lower (not more than 0.4MPa) than hard tissues (10-1500MPa) or most soft tissues (0.4-350MPa). Henceforth the development of more advanced techniques emerges to enable scaffold fabrication with improved mechanical properties (Loh and Choong, 2013).

Advanced fabrication methods undeniably have better design repeatability and part consistency, besides being able to control scaffold architecture at both macro and micro levels. However, despite possessing many leverages against conventional techniques, some drawbacks and challenges remains, such as the limitation of biomaterials that may be processed by these techniques as compared to traditional fabrication methods (Loh and Choong, 2013).