SCHOOL OF MATERIALS AND MINERAL RESOURCES ENGINEERING
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BIOACTIVE GLASS REINFORCED POLYURETHANE SCAFFOLD: EFFECT OF HARD SEGMENT CONTENT OF POLYURETHANE

By

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DECLARATION

I hereby declare that I have conducted, completed for the research work and written the dissertation entitled "**Bioactive Glass Reinforced Polyurethane Scaffold: Effect of Hard Segment Content of Polyurethane**". 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.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

3D	Three-Dimensional
ACP	Amorphous Calcium Phosphate
BG	Bioactive Glass
BD	Butanediol
DBTDL	Dibutyltin Dilaurate
DSC	Differential Scanning Calorimetry
DTG	Derivative Thermogravimetric
ECM	Extracellular Matrix
EDX	Energy Dispersive X-ray
FESEM	Field Emission Scanning Electron Microscopy
FTIR	Fourier Transform Infrared Spectroscopy
HA	Hydroxyapatite
HBSS	Hank's Balanced Salt Solution
HCAP	Carbonated Hydroxyapatite
HMDI	4,4'-Methylenebis(cyclohexyl isocyanate)
ICP	Inductively Coupled Plasma
PCL	Polycaprolactone
PSA	Particle Size Analyzer
PU	Polyurethane

THF	Tetrahydrofuran
TGA	Thermogravimetry Analysis
UTM	Universal Testing Machine
XRD	X-ray Diffraction
XRF	X-ray Fluorescence

LIST OF SYMBOLS

°	Degree
>	Greater than
<	Less than
%	Percentage
℃	Degree Celsius
wt.%	Weight Percentage
g	Gram
M	Meter
L	Liter
P	Density
MPa	Mega Pascal

PERANCAH POLIURETANA DIPERKUAT KACA BIOAKTIF: KESAN KANDUNGAN SEGMEN KERAS POLIURETANA

ABSTRAK

Penyelidikan ini memberi tumpuan kepada kesan kandungan segmen keras dalam sifat-sifat perancah komposit perancah poliuretana diperkuat kaca bioaktif. Lima jenis perancah poliuretana (PU) dengan 40-55 wt.% kandungan segmen keras mengandungi kaca bioaktif (BG) telah dihasilkan melalui kaedah garam larut lesap. PU dengan berbeza kandungan segmen keras telah dihasikan dengan dua langkah pempolimeran. Sebelum celupan dalam larutan garam seimbang Hank (HBSS) secara in vitro, perancah telah dinilai dalam sifat morfologi, struktur, fizikal, mekanikal dengan menggunakan teknik mikroskopi imbasan elektron (FESEM), analisis infra-merah (FTIR), ujian keliangan dan ujian mampatan. Perancah telah berjaya dihasilkan dengan perancah berliang melebihi 75 % dan saiz liang berada dalam julat 110-400 µm yang sesuai untuk aplikasi tisu tulang. Kekuatan mampatan bagi perancah dihasilkan berjulat antara 0.14-0.40 MPa. Eksperimen in vitro telah dilakukan untuk menyiasat bioaktiviti dan biodegradasi perancah poliuretana diperkuat kaca bioaktif yang dicelup dalam HBSS. Struktur permukaan, morfologi dan sifat-sifat kimia perancah telah dikaji dan pembentukan lapisan apatit hidrokasi (HA) di atas permukaan perancah dapat diperlihatkan. Perancah yang dihasilkan dengan kandungan segmen keras yang tertinggi menunjukkan kadar degradasi yang paling rendah. Pelarutan ion Ca dan P yang semakin menurun dalam HBSS memberi bukti lanjutan tentang kaca bioaktif telah bertindak balas dan menjadi HA.

BIOACTIVE GLASS REINFORCED POLYURETHANE SCAFFOLD: EFFECT OF HARD SEGMENT CONTENT OF POLYURETHANE

ABSTRACT

This study emphasized on the effect of hard segment content of polyurethane (PU) on the properties of bioactive glass (BG) reinforced PU. Five types of bioactive glass (BG) reinforced PU scaffolds with 40 wt.% to 55 wt.% hard segment content of PU were fabricated by salt leaching method. PU with different hard segment contents were synthesized through two-step polymerization. Before in vitro immersion in Hank's Balanced Salt Solution (HBSS), the fabricated scaffolds were characterized based on morphological, structural, physical and mechanical properties using field emission scanning electron microscopy (FESEM), fourier transform infrared spectroscopy (FTIR), porosity test and compression test. The scaffolds were successfully fabricated with more than 75% porosity with open pores of 110 – 400 μm which is suitable for bone tissue applications. Compressive strength of the scaffolds ranges from 0.14 MPa to 0.4 MPa. In vitro experiments were carried out to investigate the bioactivity and biodegradation of the scaffolds upon immersion in HBSS. The surface morphology and chemical properties of the scaffolds were evaluated and revealed the formation of hydroxyapatite (HA) layer onto the surface of the scaffolds. Scaffolds fabricated with the highest hard segment content showed the lowest degradation rate. The dissolution of decreased Ca and P ions product into HBSS confirmed that the BG reacted to form the HA layer.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Bone is known as dynamic and highly vascularized tissue that naturally capable of regeneration. It acts as a protective casting for the delicate internal organ of the body. It involved in homeostasis through its storage of calcium (Ca) and phosphorus (P) ions and by relating the concentration key electrolytes in the blood (Rahaman et al., 2011; Meng Bao et al., 2013).

Bone has high regenerative capacity especially for youngsters who do not need the major intervention such as implants for fractures to heal well. However, large bone defects from disease, infection, injury or trauma which requires treatments to promote the repair, replacement and regeneration of the defective bones. Excessive bone loss has difficulties to healing so the use of bone graft is necessary. Bone grafting is a surgical procedure that replaces missing bone with material from patient's own body, an artificial, synthetic or natural substitute. Bone graft is used as filler and scaffold to promote the bone formation and wound healing. There are many types of bone grafts based on material groups such as allograft-based, ceramic-based and polymer-based bone graft. (Stevens, 2008; O'Brien, 2011; Kumar et al., 2013).

Allograft is harvested from an individual other than the one receiving the graft. Allogenic bone is taken from a human cadaver, typically from bone bank. Allograft can only be used as temporary cover due rejection from immune system and possibility of transferring diseases from donor of patient. On the other hand, one of the most common treatment is known as autogenous bone grafting or autograft, which is transplanting tissue from one part of the body such as from iliac crest into the defect site in the same individual.

Transplanting autologous bone is more reliable and preferable because it is less likely to cause immune- and disease- related complications compared to allogenic bone and xenogeneic bone (bone from animal source). However, autograft only suitable for patient with small bone defects and poor outcomes for older patients. Moreover, harvesting autografts are highly cost, painful and associated with donor-site morbidity because of infection and hematoma (Stevens, 2008; Li et al., 2011; O'Brien, 2011; Meng Bao et al., 2013).

Over the last two decades, instead of replacing damaged tissues, the field of tissue engineering aims to regenerate them by developing biological substitutes that restore, maintain or improve tissue function (Stevens, 2008). The development of scaffolds is a main aspect in bone tissue engineering research. The scaffolds should be rigid and resilient since they function as the main supporting frame work of bone graft. Scaffolds should also be porous, biocompatible, osteoinductive (capable of promoting the differentiation of progenitor cells down an osteoblastic lineage), osteoconductive (support growth of bone and encourage the ingrowth of surrounding bone) as well as osteointegrative (integrate into surrounding bone) so that bone tissue can regenerate within the scaffolds. Furthermore, a relatively slow degradation rate is critical to provide mechanical support prior to complete native bone regeneration. (Stevens, 2008; Meng Bao et al., 2013).

Synthetic scaffolds are classified into ceramic-based, metal-based, polymer-based and composites of these. For instance, Hench (1969) invented the first bioactive glass (BG) which can survive the aggressive environment of the human body and able to form bond with bone strongly that it could not be removed without breaking the bone (Farooq et al., 2012). The basic constituents of bioactive glass are silica (SiO_2), sodium oxide (Na_2O), calcium oxide (CaO) and phosphorus pentoxide (P_2O_5), especially the 45S5

bioactive glass or 45S5 Bioglass® composition consists 45% of SiO_2, 24.5% of Na_2O, 24.5% of CaO and 6% of P_2O_5 in weight percent, it stimulates and facilitates the expression of gene to control osteogenesis and the production of growth factors (Rezwan et al., 2006). Silicon in bioactive glass helps to enhance bone mineralization and gene activation which able to enlarge the interest in substitution of silicon for calcium into synthetic hydroxyapatite (HA). When tested in vitro, three-dimensional (3D) bioglass scaffold with 70% porosity and 300-400 μm pore size exhibited hydroxyl carbonate apatite (HCA) layer on its surface that notably and significantly enhanced osteoblast activity. Hence, it is confirmed with its prospective use in bone regeneration applications (Gerhardt and Boccaccini, 2010).

Other than that, the use of biodegradable metal has widespread in biomedical applications because of its inherent strength and ductility which are important to make them appealing for hard tissue applications. For example, magnesium- (Mg-) based, titanium- (Ti-) based and iron- (Fe-) based metals have been used for bone replacement scaffold. Mg and its alloy have been selected for orthopaedic implants because of their highly supportive physical properties to human bones. Density of Mg is close to natural bones and it is biodegradable through electrochemical process, and hence it is highly potential to be used as porous biodegradable metal scaffolds (Yusop et al., 2012; Arifvianto and Zhou, 2014).

For polymer-based scaffolds, they can be natural or synthetic polymers. Commonly used natural polymers in bone tissue engineering are collagen, fibrin, silk, hyaluronic acid and chitosan whereas for synthetic polymers are poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and polycaprolactone (PCL) which can be both bioactive and biodegradable (Bose et al., 2012). Synthetic polymers are preferable because it can be produced under controlled conditions and hence show in general predictable and

reproducible mechanical and physical properties such as tensile strength, elastic modulus and degradation rate (Rezwan et al., 2006). For instance, polyurethane (PU) is widely used as long-term implant materials because it has suitable mechanical properties and excellent biocompatibility. The biocompatibility and biodegradability of polyurethane are depending on its composition and preparation method (Asefnejad et al., 2011).

However, there were problems aroused from scaffold fabricated from a single phase biomaterial therefore researches started to develop on composite scaffolds. Many of them proved that composite system of polymers and ceramics is good choice in bone tissue engineering field. For instance, Bil et al. (2010) recommended composite scaffold (45S5 bioactive glass reinforced polyurethane scaffold) consist of high porosity (more than 70%) and 100-400μm pore size as well as numerous micropores on pore walls. This PU-BG composite scaffold achieved the requirements for bone tissue engineering applications (Bil et al., 2010). They also stated that varying hard/soft segment ratio of polyurethane can be tuned to the required value for specific clinical applications. Although higher porosity scaffold has advantages on bone formation, it reduces the mechanical properties of scaffold. Hence, a balance between total porosity/pore volume and mechanical strength is very crucial for potential applications in bone tissue implants (Bil et al., 2009).

There are various methods to fabricate 3D porous polymeric scaffold such as electrospinning, 3D printing, rapid prototyping, salt leaching, thermally-induced phase separation (TIPS), gas foaming and melt moulding (Janik and Marzec, 2015). Technique is selected based on application and the pore diameters and pore shapes will be different. Each technique used to produce porous scaffold has its pros and cons. Salt leaching method is known as the common method to produce porous scaffolds especially for polymeric based scaffolds. This method is capable of controlling the composite with

specific pore size ranges and porosities by manipulating the amount and type of leaching agent. Porosity up to 93 % and pore size range of 300-500 µm can be produced by using this method (Khan and Dahman, 2012).

1.2 Problem Statement

Recently, composite scaffolds are getting more attention compare to monolithic scaffolds because the properties can be tailored to suit the complex environment of human body. Many benefits while using synthetic scaffolds because biocompatibility, versatility of chemistry, and the biological properties which are significant in the application of tissue engineering and organ substitution. It is possible to precisely control the material composition and microstructure such as porosity to obtain scaffold properties required (Boccaccini and Maquet, 2003; Dhandayuthapani et al., 2011).

Polyurethane (PU) was selected to be the matrix of composite scaffold, this is because of its unique properties such as elastic and durable, and it consists of hard and soft segments, which allow more subtle control of their structure and properties (Bil et al., 2009). Two-step polymerisation was used to synthesize PU instead of mixing all at once because it yields a more uniform distribution of hard segment sizes which in turn promotes enhance microphase separation and hence increase mechanical strength (Szycher, 1999).

Since PU scaffolds have low degradation rate, 45S5 bioactive glass (BG) was added as filler. The advantages of adding BG are improving mechanical properties of composite scaffold and increasing bioactivity, hence influence the degradation rate of PU-BG scaffolds.

Bil et al (2010) synthesized polyurethanes with different contents of hard segments and the effect of solution concentration on porosity and pore size distribution was evaluated of optimum scaffold architecture and processing route (Bil et al., 2010). Based on this study, the hard segment content influences the process of polymer coagulation as well as affecting the architecture of the scaffold. Different ratio of hard segment can be used to control its mechanical properties. (Bil et al., 2010). However, the bioactivity and biodegradation of PU-BG scaffolds with different hard segment content are yet to be discovered.

In this study, PU-BG scaffolds are developed as the promising way for bone tissue engineering. The varying of the molar ratio of hard and soft segment of PU influences the properties of the composite scaffolds because of different porosity and linkages between hard and soft segments produced.

1.3 Research Objective

In this work, the main objectives are:

i. To fabricate 45S5 bioactive glass reinforced polyurethane scaffold by salt leaching method.
ii. To study the effect of hard segment content of polyurethane on the properties of 45S5 bioactive glass reinforced polyurethane scaffold.
iii. To study the influence of hard segment content of polyurethane on degradation behaviour of 45S5 bioactive glass reinforced polyurethane scaffold.

1.4 Thesis Outline

Chapter 1 introduces on biomaterials and a novel approach for degradation behaviour of bioactive glass reinforced polyurethane scaffolds. The problem statement and objectives and general overview of this project are outlined thoroughly.

Chapter 2 provides the background and covered literature surveys and published works, closely related to the synthesis of PU and fabrication of PU-BG scaffolds as well as the effect of hard segment content to degradation behaviour of porous scaffolds.

Chapter 3 describes the general information about the materials specification, experimental procedures and characterizations used in this study.

Chapter 4 explores the discussion on the 45S5 BG prepared, PU synthesized with different hard segment contents and PU-BG scaffolds fabricated. In addition, this chapter describes the relation of the hard segment content to total porosity, mechanical strength and bioactivity and biodegradation of PU-BG scaffolds.

Chapter 5 provides a general conclusion that summarises the present research works and some suggestions for future works.

CHAPTER 2

LITERATURE REVIEW

2.1 Biomaterials

Recently, the market for biomaterials based treatment is rapidly increased (Stevens, 2008). There are many kind of bioactive materials such as bioglass, bioglass-ceramics, and calcium phosphate ceramics, have been developed. Some of them are now applied to repair and reconstruct diseased or damaged bones or tissues (ElBatal et al., 2003). Bioactive materials provide appropriate biological response and shows formation of a bond between material and the tissue. To be more specific, bioactive material defined as a material that undergoes specific surface reactions, when implanted into the body, which causes the formation of hydroxyapatite (HA) like layer that is responsible for the formation of firm bond with soft and hard tissue (De Oliveira et al., 2012).

The properties of scaffold are mainly depend on the nature of the biomaterial and the fabrication process. The nature of the biomaterial has been the subject of extensive studies including different materials such as metals, ceramics, glass, natural polymer, chemically synthesized polymer and combination of these materials to form composite such as polymer/ceramic composite. The criteria for scaffolds in bone tissue engineering have been extensively reviewed including aspects of degradation, mechanical properties, cytokine delivery and combinations of scaffold and cells (Karageorgiou and Kaplan, 2005).

2.2 Three-Dimensional (3D) Scaffold Requirements

Scaffolds provide cell attachment and migration, drug delivery and retain cells and biochemical factors. They also enable diffusion of nutrients and improve the

behaviour of cell phase mechanically and biologically in the field of tissue engineering (Chen et al., 2012; Loh and Choong, 2013). There are some basic yet important considerations while producing a variety of scaffolds. Scaffold is made to be used in living tissue, it must not cause any harmful effects to host tissues and organs, and therefore it must be biocompatible. Biocompatibility of a scaffold is vital so that it will not release toxic chemicals into the body and cause any excessive immune, inflammatory, thrombogenic or fibrogenic response and disrupt or damage an adjacent anatomic structure (O'Brien, 2011; Chen et al., 2012).

Other than biocompatibility, 3D scaffold requires biodegradability. At beginning stage of implantation, a scaffold provides temporary mechanical support to cells until the cells are able to produce extracellular matrix (ECM). A biodegradable scaffold should have retains the mechanical properties for up to 6 months for both in vivo and in vitro tissue regeneration. Depending on the medical applications of scaffold, it should be biodegradable at a controllable rate which prefer to match the rate of tissue regeneration. Moreover, the biodegraded scaffold should be non-toxic and releases out of the body without negatively influences the functions of other organs (Chu and Li, 2008; Dhandayuthapani et al., 2011; O'Brien, 2011).

A scaffold may not have to provide mechanical properties exact same as a healthy tissue, however the scaffold has to have sufficient stiffness and strength as a support and transmit force to the host tissue in the context. For instance, external and internal fixation systems might be applied to support the major load bearing force until the bone has matured (Woodruff and Hutmacher, 2010).

According to Gibson and Ashby (1999), the mechanical properties of a porous solid are based on its relative density, the properties of the material that make up the pore

edges or walls, and the anisotropic nature of the solid (Gibson and Ashby, 1999). A scaffold with high porosity has low volume fraction which means low density and consequently, low mechanical properties. Pores in a porous scaffold are defined as voids space within scaffold. Porosity and pore size are both critical factors in scaffold for tissue engineering. The size of pores in scaffold relies on the application of the scaffold. For instance, scaffolds for liver regeneration should have 20 μm of pore diameter to permit the growth of hepatocytes (Janik and Marzec, 2015). Recent researches have been studying to obtain a balance between porosity and mechanical properties of scaffold. This is because scaffolds with higher porosity may provide a greater pore volume for cell infiltration and extracellular matrix formation but lower the mechanical properties (Chu and Li, 2008).

Pores are classified into three groups and there are connected (open pores), non-connected (close pores) and combination of both. Pore interconnectivity is one of the most important factors in scaffold. Interconnecting pores (open pores) are voids linking one pore to another. A porous scaffold with non-connecting pores (closed pores) is useless and superfluous in tissue engineering. Interconnecting pore size is much more important than pore size. This is because interconnecting pores are able to provide suitably large to support and promote cell migration and proliferation in the initial stages and thus extracellular matrix infiltration of desired tissue. In tissue engineering, scaffold with 100% of interconnecting pore volume is preferable, this is because the diffusion and exchange of nutrient such as protein, oxygen and glucose and removal of waste are maximised throughout the whole scaffold pore volume (Boer et al., 2008; Loh and Choong, 2013).

2.3 Bioactive Glass (BG)

Bioactive glasses (BG) are silicate based glasses which consist of sodium, calcium and phosphate. BG shows special properties that favours in scaffold applications including osteoconduction, bonding ability between soft and hard tissues and formation of HA in biological fluid. The benefit of HA layer is able to create strong bond between bioactive glasses and human bone (Mačković et al., 2012). According to Hench (1969) who was first developed bioactive glasses stated that these glasses were able to bond tissues and safe to be used in clinical applications. There are many classes of bioactive glasses such as the conventional silicate glass (45S5 bioactive glass or Bioglass®), glass ceramic (S53P4 bioactive glass or BonAlive®) and borate based glasses (19-93B3 bioactive glass) as shown in Table 2.1 (Fu et al., 2011; Rahaman et al., 2011; van Vugt et al., 2017).

45S5 bioactive glass is the most commonly used for bone grafts. 45S5 bioactive glass composed of 24.5 wt.% Na_2O, 24.5 wt.% CaO, 45.0 wt.% SiO_2, and 6.0 wt.% P_2O_5. In less than two hours, 45S5 bioactive glass is able to form HA and binds to tissue (Farooq et al., 2012). BG with various composition are being applied in preparing of scaffolds and coating material for implants (Farooq et al., 2012).

Table 2.1: Composition 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	4.0	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	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

45S5 bioactive glass is a member of the family of silicate bioactive glass which has been clinical use since 1985. The composition of 45S5 bioactive glass (24.5 wt.% Na_2O, 24.5 wt.% CaO, 45.0 wt.% SiO_2, and 6.0 wt.% P_2O_5) as shown in Table 2.1, particularly offers an ideal environment for cell proliferation and improving differentiation of human osteoblasts to form new bone. The 6.0 wt.% P_2O_5 was added to stimulate the Ca/P constituents of HA, the inorganic mineral phase of bone (Rezwan et al., 2006).

45S5 bioactive glass has high bioactivity by producing rapid regeneration of trabeculant bone with an amount, architecture and bio-mechanical quality of bone that fit the original site. Osteostimulation and osteoconduction is a combination of processes that cause the rapid regeneration of bone. With such composition (less than 55 % SiO_2) and both osteostimulative and osteoconductive, the bioactivity of 45S5 bioactive glass is grouped in region A (rapid reaction on bioactive glass surface/ high bioactivity index) as shown in Figure 2.1. For region B, glasses are approximately inert after implantation and only exhibit osteoconductivity such as HA as whereas compositions in region C are resorbed between 10-30 days in tissue and last region is region D, where the compositions are not technically practical. Lastly, 45S5 bioactive glass fulfilled the requirements of

scaffold included bioactive, biocompatible, osteoconductive, nontoxic and noninflammatory and lastly, nonimmunogenic agent. Because of these requirements, 45S5 bioactive glass provides an ideal environment for proliferation and differentiation of osteoblast to form new bone that has a strong mechanical bond to the surface of implants (Verrier et al., 2004; Hench, 2006, 2013; Rezwan et al., 2006; Chu and Li, 2008; Gerhardt and Boccaccini, 2010; Ryszkowska et al., 2010).

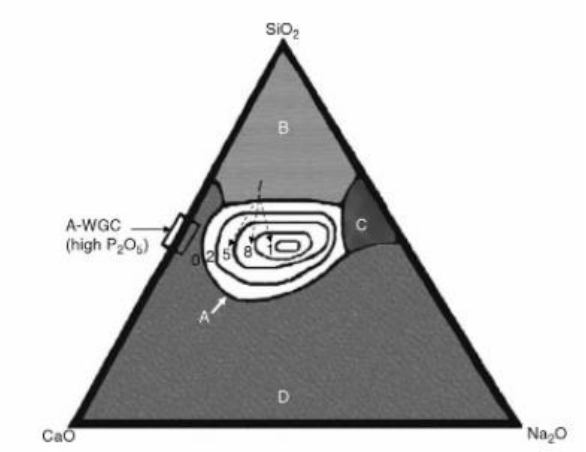

Figure 2.1: Compositional dependence (in wt. %) of bone bonding and soft tissue bonding of bioactive glasses and glass-ceramic (Chu and Li, 2008).

2.3.1 Mechanism of Bioactivity and Bone Bonding of 45S5 Bioactive Glass

The mechanism of bioactivity and bone bonding of 45S5 bioactive glass have been widely studied and analysed. According to Rahaman et al. (2011), the bonding of this bioactive glass to bone has been attributed to the formation of carbonate-substituted hydroxyapatite-like (HCA) layer on the glass surface while in contact with body fluid. This HCA layer is similar to the mineral constituent of bone which able to bond firmly with living bone and tissue (Rahaman et al., 2011). As described by Hench (2013), the mechanism of bioactivity of 45S5 glass is explained in five stages (Hench, 2013).

At the beginning stage, the rapid ion exchange reactions between the glass network modifiers (Na^+ and Ca^{2+}) with H^+ ions from the solution, leads to hydrolysis of the silica groups and the creation of silanol (Si-OH) group on the glass surface:

$$Si - O - Na + Na^+ + H^+ \rightarrow Si - OH + Na^+ (aq)$$

The pH of the solution increases due to the consumption of H^+ ions.

Next, because of the increase in pH, it attacks the SiO_2 glass network and the dissolution of silica, in the form of silicic acid, $Si(OH)_4$, into the solution and the continued formation of Si-OH groups on the glass surface:

$$Si - O - Si + H_2O \rightarrow Si - OH + OH - Si$$

While the solubility of silica is low, the products of 45S5 glass and glass-ceramic dissolution in aqueous solutions have an increase in Si concentration, showing that the dissolution of silica is an important mechanism. But, other mechanism could also contribute to the increase in Si concentration (Rahaman et al., 2011).

In stage 3, condensation and polymerization of an amorphous SiO_2-rich layer with normally 1-2 micron thickness on the surface of the glass depleted in Na^+ and Ca^{2+} happens. In stage 4, further dissolution of the glass, coupled with migration of Ca^{2+} and $(PO_4)^{3-}$ ions from the glass through the SiO_2-rich layer and from the solution, leading to the formation of an amorphous calcium phosphate (ACP) layer on the surface of the SiO_2-rich layer (Rahaman et al., 2011).

In stage 5, the glass continue to dissolve as the ACP layer incorporates $(OH)^-$ and $(CO_3)^{2-}$ ions from the solution and crystallizes as an HCA bi-layer on glass surface. With the initial formation of an HCA layer, the reaction layers promote adsorption and desorption of growth factors (stage 6), and affect the duration macrophages are needed to

prepare implant site for tissue repair (stage 7) followed by attachment (stage 8), proliferation and differentiation of osteo-progenitor cells which improve bone regeneration (stage 9). Osteoblast (bone-forming cells) create extracellular matrix (collagen) which mineralizes to form a nanocrystalline mineral and collagen on the glass surface when the degradation (stage 10-11) and conversion of the glass continue over time (Hench, 2006; Gerhardt and Boccaccini, 2010; Rahaman et al., 2011).

After implantation, 45S5 bioactive glass undergoes chemical degradation, releasing ions such as Na^+ and Ca^{2+}, and conversion to an HCA material. Si appears in the form of silicic acid, $Si(OH)_4$, is also released during the degradation by dissolution or by other mechanisms, such as small pieces of silica-rich material eaten by phagocytes and excreted out (Rahaman et al., 2011). Figure 2.2 shows the sequence of interfacial reactions which involved formation a bond between a bone and a class A bioactive glass (Gerhardt and Boccaccini, 2010).

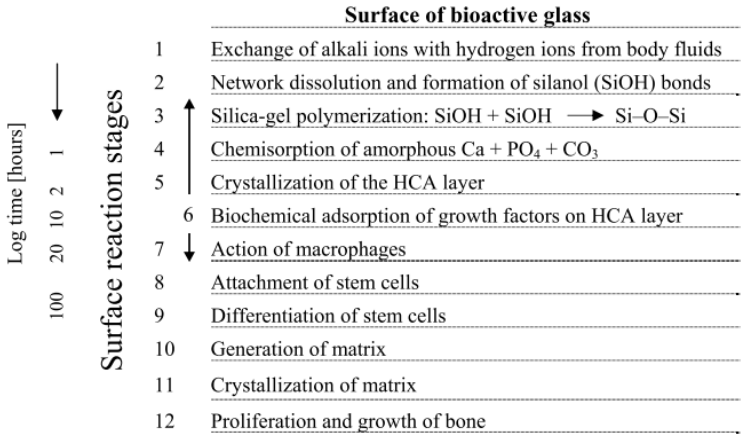

Figure 2.2: Sequences of interfacial reaction which involved formation a bond between a bone and a class A bioactive glass (Gerhardt and Boccaccini, 2010).

2.3.2 Advantages and Disadvantages of Bioactive Glass (BG)

The benefit of using bioactive glasses (BG) is ease in controlling chemical composition and hence the degradation rate which make them attractive as scaffold materials. By changing either composition or thermal or environmental processing history, the structure and chemistry of glasses can be made over a wide range. Therefore, it is possible to design glass scaffold with different degradation rates to match that of both ingrowth and remodelling (Fu et al., 2011). In short, 45S5 bioactive glass has excellent osteoconductivity and bioactivity, controllable biodegradability and ability to induce osteogenesis and angiogenesis (Ryszkowska et al., 2010).

However, BG have low fracture toughness which restrict the application of bioactive glasses as scaffold structure in load-bearing situations. The crystallization of BG will lower the bioactivity level and even turned it into an inert material. Therefore, bioactive glass reinforced polymer scaffold was developed and being known as a strategy to improve the mechanical behaviour of bioactive-glass based materials (De Oliveira et al., 2012).

2.4 Polymer Scaffolds

Polymers have been widely used as biomaterials for the fabrication of tissue engineering scaffold. In biomedical applications, the materials were selected based on their material chemistry, molecular weight, solubility, shape and structure, hydrophilicity/hydrophobicity, lubricity, surface energy, water absorption degradation, and erosion mechanism (Nair and Laurencin, 2007). Because polymeric scaffolds have unique properties, therefore they draw a great attention. The unique properties are high surface to volume ratio, high porosity with small pore size, physical properties (such as mechanical properties) and biodegradation. Moreover, they have many advantages

included biocompatibility, versatility of chemistry, and the biological properties which are significant in the application of tissue engineering and organ substitution (Dhandayuthapani et al., 2011).

Both natural occurring polymers and synthetic biodegradable polymers have been investigated as biodegradable polymeric biomaterials. Examples of natural occurring polymers are chitin, chitosan, elastin, alginate, collagen and silk. Collagen can be extracted from animal tissues and produced thru recombinant technology. It has high mechanical properties, bioactivity and osteoconductive properties. However, the downside of collagen are including difficulty in processing and control the extent and degradation rate (Patel et al., 2011). For chitosan, it is able to support the attachment and expression of ECM component by chondrocytes. Collagen and chitosan have been widely investigated for bone engineering because of apparent osteoconductive properties and able to promote the differentiation to osteoblast (Chu and Li, 2008). Natural based polymer are biocompatible and enzymatically biodegradable.

Despite that natural occurring polymers have many advantages, synthetic polymers offer more advantages and this is because of their off-the-shell availability as well as biocompatibility and biodegradability. Furthermore, they can be produced under controlled conditions and hence show in general predictable and reproducible mechanical and physical properties such as tensile strength, elastic modulus and degradation rate. The possibility of toxicity, rejection from immune system and infections are lower for pure synthetic polymers with constituent monomeric units having simple structure (Rezwan et al., 2006). Polyester is the most common synthetic polymer. Poly(glycolic acid) (PGA), poly(L-lactic acid) (PLLA), poly(D,L-lactic acid-co-glycolic acid) (PLGA) and poly(ε-caprolactone) (PCL) are type of polyester. There are other type of synthetic polymers

including poly(ethylene glycol) (PEG), polyoethoester (POE) and polyurethane (PU) as shown in Table 2.2 (Narayan, 2009).

Table 2.2: Synthetic polymers and their properties (Narayan, 2009).

Synthetic Polymer	**Curing Method**		**Degradation Method**	**Degradation Product**
	Crosslinking	**Entanglement**		
PGA		✓	Ester hydrolysis	Glycolic acid
PLLA		✓	Ester hydrolysis	Lactic acid
PLGA		✓	Ester hydrolysis	Lactic acid and glycolic acid
PCL		✓	Ester hydrolysis	Caproic acid
PEG		✓	Nondegradable	Not applicable
POE		✓	Ester hydrolysis	Various acid depending upon R group
PU	✓		Ester, urethane or urea hydrolysis	Diisocyanate and diols

2.4.1 Biodegradable Polyurethane (PU)

Polyurethane (PU) is a class of polymers that consist of the urethane (-NH-CO-O-) linkage that is typically generated through the addition of an isocyanate to a hydroxyl group (Patel et al., 2011). PU is an elastomeric polymer that is typically non-degradable (Table 2.2), but the positive attributes including flexible mechanical strength and biocompatibility, have led to the synthesis of degradable polyurethanes with non-toxic diisocyanate derivatives (Narayan, 2009). For instance, PU scaffold that has elastomeric properties are suitable to be used in cardiac applications because it can support cardiac function (Sin et al., 2010).

Ayres developed biodegradable and aqueous dispersible polyurethane (PU) through prepolymer mixing process, using polycaprolactone-diol (PCL), 2.2-

bis(hydroxymethyl) propionic acid (DMPA) and isophorone diisocynate. The chains of the PUs are extendable by silane groups by the reaction of the prepolymer with aminosilanes. Crosslinking reactions occur to form a stable siloxane-linked network that is able to improve the properties of PU during water evaporation. Hydrolysed silanes can be added to the PU to increase the adhesion with inorganic particles (De Oliveira et al., 2012).

PU contains hard and soft segments which allow more subtle control over the structure and properties. The hard and rigid segments are produced from the reaction between diisocyanate and the chain extender, whereas the soft segments are polyether, polyester or polycarbonate diol. The hard segment of PU influences the degree of phase separation, then affects the physical and mechanical properties, degradation rate and biocompatibility. Variation in this segments, properties of PU can be used in many areas of tissue engineering which included reconstruction of soft tissue or for cartilage and bone regeneration (De Oliveira et al., 2012; Kumagai et al., 2017).

The properties of PU depends on the monomer used and preparation method. There are many methods can be used to prepare PU, including prepolymerization, single step polymerization and quasiprepolymer. For prepolymerization, firstly, diisocyanate reacts with the soft segment polyol to form the prepolymer and hence, the characteristic urethane linkages are formed through the reaction between the isocyanate groups and the hydroxyl terminated end groups of the polyol as shown in Figure 2.3. The following step is the low molecular weight chain extender is used to link the prepolymer segments yielding a high molecular weight polymer. A diol reacts with –NCO-terminated prepolymer in the chain extension reaction step, urethane linkage will be formed, on the other hand, a diamine involves in the reaction with prepolymer, because of the reaction between $–NH_2$ groups and –NCO terminated prepolymers, urea linkages will formed

(Figure 2.3) (Cauich-rodríguez et al., 2012; Wong and Badri, 2012; Zafar and Sharmin, 2012).

Single step polymerization involves diisocyante, polyol and catalyst are mixed together in one step and this method is suitable to be used to produce thin wall products because of heat liberated. Last method is quasiprepolymer which involves 2 steps polymerization as well, prepolymers are normally produced with a mole ratio of 2 moles of diisocyanate to 1 mole of polyol. If the mole ratio of diisocyanate to polyol is higher such as ratio 3:1, the resultant product is called a quasiprepolymer. As the reaction proceeds, the chain length of prepolymer will increase as the hydroxyl groups react with the terminal NCO end groups of the prepolymers (Wong and Badri, 2012). PU is commonly prepare in two steps because better control of structure of PU and produce a more uniform distribution of hard segment sizes which in turn promotes enhance microphase separation and hence increase mechanical strength (Szycher, 1999).

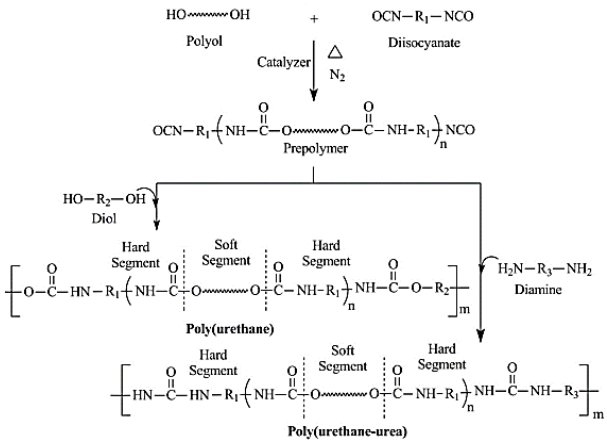

Figure 2.3: Standard two-step reaction to prepare segmented poly (urethane)s and poly(urethane-urea)s (Cauich-rodríguez et al., 2012).

2.5 Bioactive Glass Reinforced Polyurethane (PU-BG) Scaffold

The concept of bioceramic reinforced biopolymer scaffolds started by Bonfield et al. (1981). They described that the significant constituent are collagen and hydroxyapatite therefore, bone may be considered as HA reinforced collagen composite. The role of HA is the major load bearing component of bone whereas collagen acts as matrix to HA. With the presence of collagen, it minimize the potential of brittle fracture of ceramic (HA) structure itself. They made use of synthetic polymer, polyethylene as matrix and hydroxyapatite powder as filler to replace bone tissue (Bonfield et al., 1981).

Composite scaffolds are getting more attentions by combining the advantages of both polymer and ceramic scaffolds to meet the requirements of mechanical and physiological of host tissue. The properties of BG including bioactive, biocompatible, osteoconductive and nontoxic made it as an ideal filler in biopolymer composite scaffolds (Rezwan et al., 2006; Ryszkowska et al., 2010).

PCL is a thermoplastic polyester elastomer which degrades very slowly due to its hydrophobicity. As explained by Zeimaran et al. (2015), mechanical properties of PCL-based scaffolds are influenced by the addition of BG particle size, composition and fabrication method. Elasticity of scaffolds decreased with increased filler content due to agglomeration. Still the weight loss for composites is relatively higher than unfilled polymer because bioceramic such as BG in fluid ingress into the bulk of sample and bioactive glass dissolution (Zeimaran et al., 2015).

PU is also a thermoplastic polyester elastomer that has segmented structure and slow degradation compare to other synthetic polymer therefore it is popular for hard tissue implant. Ryszkowska, Auguscik and Sheikh (2010) confirmed PU-BG scaffolds have high bioactivity based on the behaviour of these composite scaffolds in SBF leading the

formation of HA on the surfaces of scaffolds. They synthesis PU from HMDI, PCL and ethylene glycol with different molar ratios. BG containing scaffolds undergo bulk degradation through hydrolysis of ester bonds, showing that PU soft segments are more susceptible to degradation compared to hard segment (Ryszkowska et al., 2010).

2.6 Technique to Fabricate Porous Scaffold

There are many techniques developed to produce porous scaffolds and divided into two categories, non-designed manufacturing techniques and designed manufacturing techniques. For non-designed manufacturing technique, there are emulsion freeze-drying, gas forming, thermally induced phase separation, solvent leaching and combination of these techniques. Rapid prototyping of solid free form technologies is one of the designed manufacturing technique (Sultana et al., 2015).

For emulsion freeze-drying technique (Figure 2.4), a homogeneous (of 2 immiscible phases) emulsion is rapidly frozen in liquid nitrogen to maintain liquid state structure and then freeze-dried to create a porous scaffold with pore size with the range of 20 to 200 μm and above 90 % porosity. This method is mainly used for the fabrication of soft tissue scaffolds and it can reduce toxic solvent and time for leaching process of porogen components. However, the instability of emulsion needs the addition of suitable surfactants such as waterborne polyurethane and complicated synthesis process.

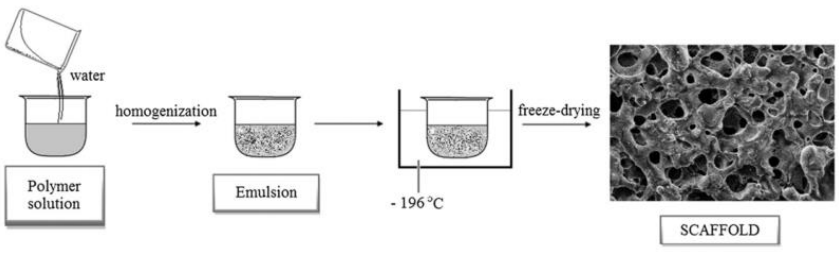

Figure 2.4: Scaffold fabrication using emulsion freeze-drying technique (Janik and Marzec, 2015).

Other than emulsion freeze-drying, gas foaming is one of the methods that is used to fabricate porous scaffold. This method involves releasing gas as a product of the thermal degradation of gas forming agent. Gas is used as porogen so it does not involve leaching processes. This method is rarely used because of difficulty in controlling the pore diameter and average of pore diameter is too big and unconnected to allow adequate cell proliferation (Janik and Marzec, 2015; Sultana et al., 2015).

Next technique is thermally induced phase separation (TIPS) (Figure 2.5), it is a process that depending on the thermodynamics and kinetic behaviour of the polymer solution under certain conditions. TIPS is involving quenching polymer solution below solvent crystallization temperature/ freezing point and liquid-liquid phase separation temperature to form two different phases and there are rich and poor phases. Poor phases will crystallize and being removed to create a porous scaffold (Janik and Marzec, 2015; Sultana et al., 2015).

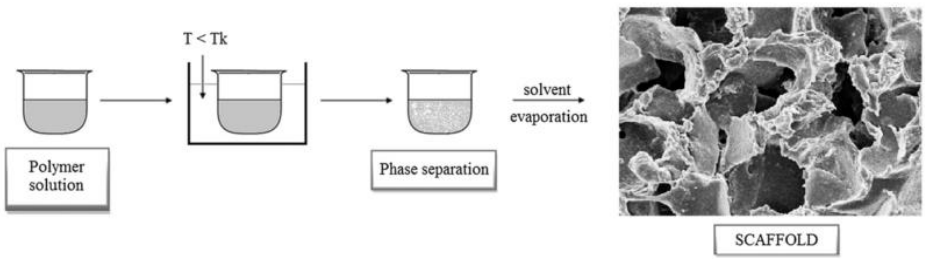

Figure 2.5: Scaffold fabrication using TIPS (Janik and Marzec, 2015).

One of the most common methods to produce scaffold is salt leaching technique. It uses inorganic salt particles, paraffin and gelatin or ice as pore forming agent.

According to Janik and Maezec (2015), the shape and size of pores are depending on the shape and dimensions of leachable particles used (Janik and Marzec, 2015). Therefore, salt leaching technique is simple yet able to control the pore size and porosity by changing the size and amount of leachable particles (Bil et al., 2009). No specialized equipment needed if using this technique.

Porous construct of synthetic biodegradable polymer scaffold is able to be produced with specific surface to volume ratio, crystallinity, pore size and porosity by using this technique (Sultana et al., 2015). However, this technique is not suitable for thick materials because of difficult in leaching the particles/salt from large volume. Salt leaching is the most suitable method to fabricate bioactive glass reinforced polyurethane (PU-BG) scaffold in this project (Zeimaran et al., 2015).

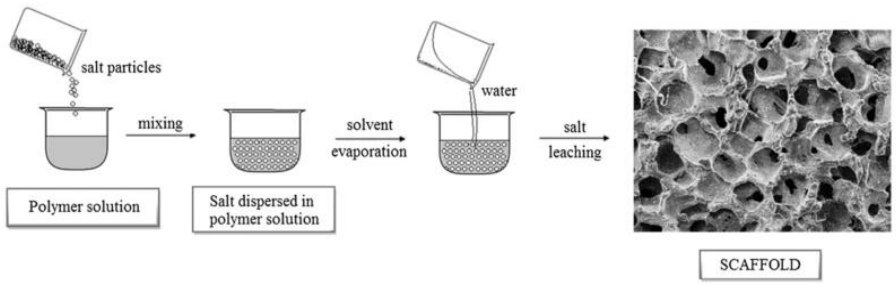

Figure 2.6: Scaffold fabrication using salt leaching technique (Janik and Marzec, 2015).

2.7 Factors affecting Degradation Behaviour of Porous Scaffold

According to Pan and Din (2012), for a tissue engineering material, it is very important to know the degradation of a porous scaffold. This is because it not only affects cell viability and cell growth, it even host response in engineering a tissue. They reported that there are many factors will influence scaffold degradation, such as porosity and pore size, composition and mechanical loading (Pan and Ding, 2012).