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EFFECT OF SALT CONCENTRATION ON MORPHOLOGY AND MECHANICAL PROPERTIES OF POLYURETHANE/BIOGLASS COMPOSITE SCAFFOLD

By

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DECLARATION

I hereby declare that I have conducted, completed for the research work and written the dissertation entitled "**Effect Of Salt Concentration on Morphology and Mechanical Properties of Polyurethane/Bioglass Composite Scaffold**". 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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LIST OF ABBREVIATIONS

PU	Polyurethane
BG	Bioactive Glass
Al_2O_3	Aluminium oxide
CaO	Calcium oxide
Na_2O	Sodium oxide
SiO_2	Silicon dioxide
P_2O_5	Phosphorous pentoxide
THF	Tetrahydrofuran
XRD	X-Ray Diffraction
FTIR	Fourier-Transform Infrared Spectroscopy
FESEM	Field Emission Scanning Electron Microscopy
XRF	X-Ray Fluorescence
PSA	Particle Size Analyzer
TG/ DTG	Thermogravimetry
DSC	Differential Scanning Calorimetry
HA	Hydroxyapatite
3D	Three-Dimensional

LIST OF SYMBOLS

mm	Milimeter
ml	Millileter
cm	Centimeter
Tg	Glass transition temperature
Tm	Melting temperature
wt. %	Weight percent
λ	Wavelength
µm	Micrometer

KESAN KEPEKATAN GARAM TERHADAP MORFOLOGI DAN SIFAT MEKANIKAL PERANCAH POLIURETANA/BIOKACA

ABSTRAK

Tujuan kejuruteraan tisu adalah untuk menghasilkan perancah tiga dimensi yang boleh digunakan untuk pembinaan semula dan regenerasi tisu dan organ yang rosak. Pelbagai jenis teknik telah dibangunkan untuk menghasilkan sama ada perancah berserabut atau poros dari polimer, logam, tekstil komposit dan seramik. Walau bagaimanapun, bahan yang paling baik adalah polimer biodegradasi kerana sifat mekaniknya yang komprehensif, keupayaan untuk mengawal kadar degradasi dan persamaan dengan struktur tisu semulajadi. Dalam kajian ini, perancah komposit poliuretana bertetulang kaca bioaktif berliang dengan ratio garam yang berbeza dibuat dengan menggunakan teknik pelarut garam kerana kaedah ini tidak melibatkan proses suhu tinggi yang akan mempengaruhi sifat-sifat polimer. Mikrostruktur perancah komposit menghasilkan keliangan yang saling berkait rapat, sesuai untuk pertumbuhan semula tulang dan pembentukan saluran darah dan kapilari dalam tisu hidup. Salah satu faktor yang menentukan keliangan adalah perancah. Kepekatan garam adalah salah satu faktor yang mempengaruhi keliangan. Sifat perancah telah dikaji dengan menggunakan ratio perencah:garam yang berbeza. Kepekatan garam yang berbeza telah digunakan dengan tujuan untuk mengoptimumkan rangkaian liang dan untuk mendapatkan perancah dengan sifat mekanikal yang optimum. Ciri-ciri kimia dan fizikal dari perancah berliang yang dihasilkan telah diperiksa oleh beberapa teknik pencirian termasuk Mikroskopi Elektron Penskanan (SEM), Analisis Termogravimetri (TGA), Analisis Infra-merah (FTIR), dan ujian kekuatan mampatan. Secara keseluruhan, perancah komposit dengan nisbah garam 1: 3 mempunyai liang yang diedarkan secara seragam dan mempunyai kekuatan mampatan yang paling tinggi.

EFFECT OF SALT CONCENTRATION ON MORPHOLOGY AND MECHANICAL PROPERTIES OF POLYURETHANE/BIOGLASS COMPOSITE SCAFFOLD

ABSTRACT

The aim of tissue engineering is the fabrication of three-dimensional scaffolds that can be used for the reconstruction and regeneration of damaged tissues and organs. A broad variety of techniques have been developed to create either fibrous or porous scaffolds from polymers, metals, composite textiles and ceramics. Nevertheless, the most promising materials are biodegradable polymers due to their comprehensive mechanical properties, ability to control the rate of degradation and similarities to natural tissue structures. In this study, porous bioactive glass (BG) reinforced polyurethane (PU) composite scaffolds with different salt ratio was fabricated by using salt leaching technique as this method does not involve any high temperature process that will affect the properties of the polymer. One of the factors that determine the properties of the scaffold is the porosity. Salt concentration is one of the parameters that influence the porosity. Various salt concentration were used with the aim of optimizing the pore network and to obtain the optimum mechanical properties of scaffolds. The chemical and physical properties of the fabricated porous scaffolds were examined by several characterization techniques including Scanning Electron Microscopy (SEM), Thermogravimetric Analysis (TGA), Fourier Transform Infrared Spectroscopy (FTIR), and compressive strength test. Composite scaffolds with low amount of porosity shows a better compressive strength and modulus. Overall, composite scaffolds with salt ratio 1:3 consists of pore which was homogeneously distributed and have the highest compressive strength.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Bone is a dynamic tissue and it undergoes continuous transformation through the lifetime of an individual also it involves in movement with load bearing role and protects delicate vital organs of the body (Saravanan et al. 2016b). Bone tissue engineering targets is to improve musculoskeletal health by preparing a living bone graft substitute to fill and assist in repairing the bone defects caused by disease, trauma, or congenital malformations or to augment bone stock around an implant site (Tetteh et al. 2014). It involves the engineering disciplines and principles of bone biology to augment bone loss by the use of temporary matrices which known as scaffolds.

Between the many tissues in the body, bone has the highest possible for regeneration and therefore is a model of prototype for the enunciation of principles of tissue engineering in general. Tissue engineering is a rising science technology and can be useful to improve the various clinical situations, including joint replacement, spinal fusion, and fracture nonunion and pathological loss of bones (Sabir et al. 2009). There are three key ingredients for tissue engineering and tissue regeneration which is signals, stem cells and scaffolding. Tissue morphogenesis and inductive cues will influent the specificity of signals in the embryo and they are generally recpeated during regeneration.

For first generation, orthopedic implant materials were choose because of their biomechanical properties for structural renovation. Later, new renovation was done in second generation which the bone implant materials were engineered to be bioresorbable or bioactive to encourage and boost up the tissue growth. At this level, the development

involved with the development of tissue engineering scaffolds as cell supports for various types of tissue. Nowadays, bone implant materials are designed to encourage the formation of bone and a lot of bone graft substitute materials. It is also been applied as experimental scaffolds to encourage cells for bone tissue engineering. Bone defect repair using the tissue engineering approach is perceived as a better approach because the repair process may proceed with the patient's own tissue by the time the regeneration is complete (Manuscript 2013).

The main role of bone tissue engineering is for providing structural support for human body. Autologous bone grafting is the common clinical standard for bone defect healing and nonunion. This treatment presents serious problems with donor site morbidity, prolonged operation time and the limited accessibility of graft materials (Zeimaran et al., 2015). But, mostly the clinical treatments of bone defects caused by trauma, cancer, infection, or congenital deformity were repaired by the application of autograft and allograft.

An autograft is a tissue or bone that is taken from a part of a person's own body and transferred into another while allograft is a organ or tissue that is transferred from one person to another. But, there are some disadvantageous of using autograft which is the need for a secondary surgery, donor shortage and donorsite morbidity. Allografts can be used as alternative but there are some limitations which is immune rejection and risk of disease transmission. (Kroeze et al. 2009).

Bone tissue engineering have its own aim which is restore function of diseased or damaged bone tissue by combining functional cells and isolated biodegradable scaffolds made up from engineered biomaterials. In bone tissue engineering, the most important application of scaffolding materials are bioactive glass and related bioactive composite materials (Rezwan et al. 2006). Bioactive glasses have its own disadvantage which it can

encourage more bone regeneration than other bioactive ceramics. Most of bioactive glass compositions have high bioactivity and good compatibility.

Professor Hench developed the first bioactive glass, containing 45 wt % SiO_2, 24.5 wt % Na_2O, 24.4 wt % CaO and 6 wt % P_2O_5, also known as 45S5 bioglass which formed a strong interfacial bond to bone (Elgayar et al. 2005). The formation of a hydroxycarbonate apatite layer on the surface shows the bioactivity. Also, the rate of hydroxycarbonate apatite (HCA) formation affect the rate of tissue bonding appears, which is thought to be produced by a reactions between the implanted materials and the surrounding tissues. Bioglass 45S5 making strong bonds with bone where it is very hard to remove the bone.

Besides, in tissue engineering application, biodegradable polymer scaffolds have played an important part in wide range from the last decade such as bone scaffolds. Polymer materials are more advantageous than other material because of their biocompatibility, degradation rate, mechanical properties, and composition of scaffold polymer materials can be controlled precisely (Sabir et al. 2009). Polymers plays an important role for the fabrication of medical device and tissue-engineering scaffold.

Composition, structure, and arrangement of their constituent macro-molecules will affect the properties of polymers depend. There are three main types of polymers used as biomaterials which is naturally occurring polymers, synthetic biodegradable, and synthetic nonbiodegradable polymers. Natural materials have better interactions with the cells which encourage them to increase the cells' performance in biological system (Dhandayuthapani et al. 2011). While for synthetic biomaterial, it may ease the restoration of structure and purpose of damaged or diseased tissues. Synthetic polymers is very useful in biomedical field because of their properties such as porosity and mechanical characteristics can be used for specific applications.

Polyurethane (PU), a synthetic polymer which have unique segmented structure with combination of appropriate raw materials and additives will produce more diverse properties. PU contain of wide range of physical and mechanical properties, from thermoplastic to thermosetting, from hydrophobic to hydrophilic, from stable to degradable materials depends on the composition and synthesis procedure used (Janik & Marzec 2015). PUs shows moderate compatibility with blood, and are characterised by bioresorbability, biocompatibility, and excellent mechanical properties, which can be adjusted to specific tissue. The words biocompatibility and biomaterial is to indicate the biological performance of materials. Also, development of polymer/bioactive glass will be the strategy to increase the mechanical performance of bioactive glass-based materials.

1.2 Problem Statement

Polyurethane is selected as a tissue engineering composite matrix of bone tissue due to its power to perform magnificently in a large diversity of applications in industries, especially in medical implants. Properties such as shear strength, compression strength, wear resistance and ductility that required for each application is varied. Mechanical properties such as wear resistance and compression strength for polyurethane shows good performance during servicing for most of the applications stated above.

But, there is a problem with polyurethane because the bioactivity is low because fully reacted polyurethane is a chemically inert polymer. So, addition of 45S5 bioactive glass can improve the bioactivity of polyurethane. This is because the 45S5 bioactive glass has relatively high bioactivity towards bone tissues. Besides, 45S5 bioactive glass is also a high hardness glass product but low fracture resistance. Thus, the strength and

bioactivity of polyurethane scaffolds can be improved by reinforced with 45S5 bioactive glass filler.

There are some requirements in producing scaffold which is the porous scaffold foam should be higher to assign cell attachment thus the tissue can growth. In this experiment, the technique used in producing the porous scaffold is by using salt leaching technique. This technique has been used because of easy fabrication and do not need specialized equipment. Difference of salt concentration may change the pore structure of the scaffolds.

In order to create the porous structure, the salt concentration plays a significant effect on the properties of the scaffold. Porosity and pore size of the scaffold play a crucial role in determining the mechanical properties as well as to induce tissue ingrowth and bone formation. Hence, it is important to study and determine the optimum salt concentration to produce scaffold with desired mechanical properties and acceptable morphology.

1.3 Research Objectives

The main objectives of these work are:

i) To fabricate PU-BG scaffold by using salt leaching technique.

ii) To study the effect of salt concentration on porosity and mechanical properties of the scaffold.

1.4 Scope of Research

In this study, melt quench bioglass will be used as filler to reinforce polyurethane scaffold which will be fabricated by using salt leaching technique. Pores with greater diameters will have a lower total porosity and higher mechanical strength (Janik, 2015). Furthermore, high porosity and high interconnectivity are required to minimise the amount of implanted polymer and to increase the specific surface area for cell attachment and tissue ingrowth, facilitating a uniform distribution of cells and adequate transport of nutrients and cellular waste products (Hou et al., 2003). If the amount of salt added is high, then a deficient structure with voids will be formed due to close geometric packing. Meanwhile, if the salt added is not sufficient, then the polymer solution will surround the particles and isolated pores will appear.

Several characterization methods have been done on each porous scaffolds samples fabricated in order to observe their respective properties. The microstructure and surface morphology of porous scaffolds was observed by using Scanning Electron Microscopy (SEM). The thermal properties of scaffolds was characterized by using Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC). Dynamic Mechanical Analysis (DMA) was performed to understand the effect of BG particles towards the modulus of PU scaffolds. For the extent of bonding groups intensity present in the scaffolds sample is carried out by using Fourier Transform Infrared Spectroscopy (FTIR). The total porosity of scaffolds sample prepared is determined by using Gravimetric technique.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

An important tissue, the bone, not only plays an important role in movement and in the protection and support of other organs, but also plays an important role in controlling critical human physiological functions, including mineral storage, blood cell formation, homeostasis, and blood pH regulation (Farokhi et al., 2016). The primary purpose of bone tissue engineering is to restore and maintain functionality of harm or diseased bone tissues by means of a synergic combination of cell biology, materials science and engineering (Fabbri et al., 2010). Nowadays, the most transplanted tissue is bone with an incidence of nearly 15 million fracture cases per year.

The main objective of tissue engineering is to fabricate functional replacement for broken tissues or organs. In addition, it must act as the basecoat that allows cells to attach, reproduce, differentiate and organize into normal, healthy bone as the scaffold degrades (Jones, 2013). It is also based on the studies of bone mechanics, bone structure, and tissue formation to induce new functional bone tissues. In tissue engineering, the main important thing is scaffold where it represents an alternative to conventional implantation of organ and tissues. In the context of bone tissue engineering, a scaffold is the extracellular matrix (ECM) of bone, which means it is the unique microenvironmental niche for bone morphogenesis. The basic of tissue engineering for tissue induction responding to stem cells is scaffolding of extracellular matrix with the triad of signals (Reddi 2007). Figure 2.1 shows an illustration about how the process of bone generation had been done by using scaffolds.

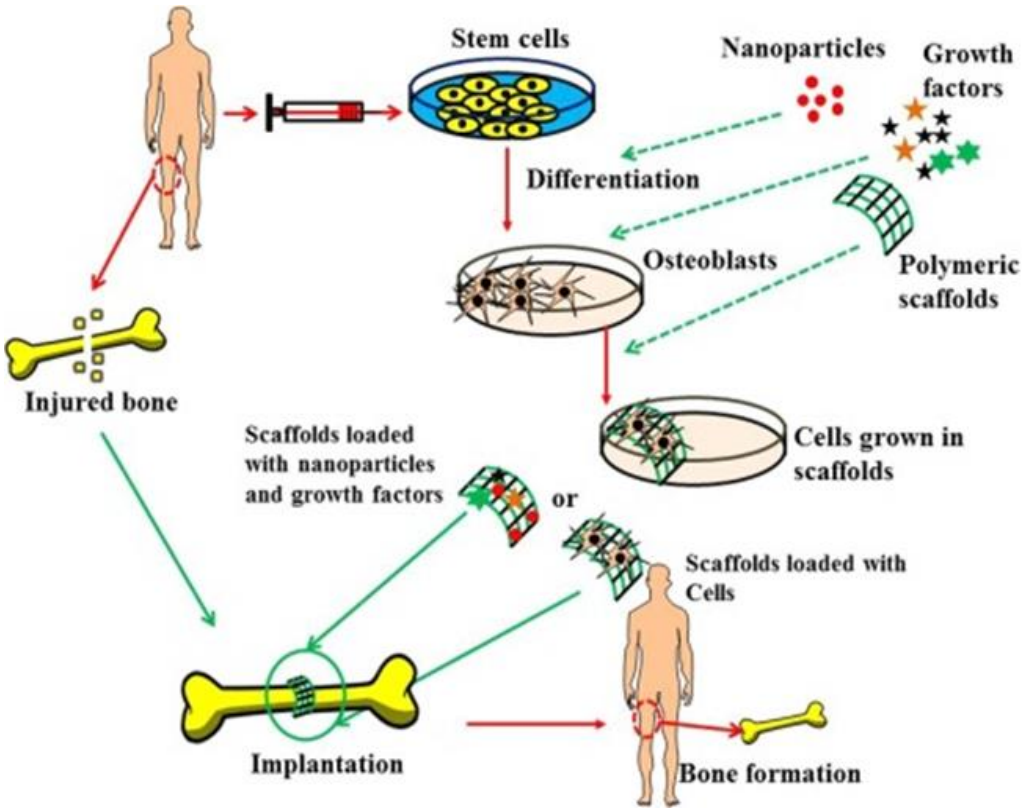

Figure 2.1: Bone tissue engineering to treat critical sized bone defects (Saravanan et al. 2016)

Scaffolds have some important role in providing support for cells to proliferate and maintain the differential function of replacements for impaired organs or tissues. The scaffolds have its primary goal which is providing appropriate base for tissue growth and cell proliferation (Janik and Marzec, 2015). Moreover, scaffold architecture gives the ultimate shape of the new bone and cartilage (Tajbakhsh, 2017). According to W.Khan et al. (2012), for bone formation, the scaffolds are required to be large and interconnected pores (which make it easy for cell infiltration and matrix deposition) and rough inner surfaces (which facilitate cell attachment), which produce from osteoconductive materials such as bone protein and hydroxyapatite, and with mechanical properties same with those of native bone (both to allow load-bearing and stimulate osteogenesis).

Besides, there two main function of scaffold which is biological and structural. For biological, the function is to encourage the attachment, growth and proliferation of cells. While, the function of structural are to fill tissue defects and provide a biocompatible template leading for the ingrowth of cells, newly formed tissues, blood vessels and the maintenance of extracellular matrix. An ideal scaffold should act as a biocompatible template for osteoprogenitor cell growth and assist in the differentiation of mesenchymal stem cells into osteoblasts, as well as aid the production, organization and maintenance of an extracellular matrix (Gogolewski, 2007).

2.2 Selection of materials in tissue engineering

There are some characteristics need to be considered in producing scaffolds. First of all, the scaffold must have structure that is three-dimensional and highly porous with an interconnected network for cell growth and flow transport of nutrients and metabolic waste. The surface chemistry must be appropriate for cell attachment, proliferation and differentiation. Other characteristic that need to be considered is the scaffold need to be bioresorbable and biocompatible with controllable degradation and resorption rate matching cell/tissue growth in vitro/in vivo. Besides, the mechanical properties must be suitable to match those of the tissues at the site of implantation. The scaffold must have an interconnected porous structure with porosity > 90% and diameters between 300-500 μm for tissue ingrowth and vascularization, cell penetration, and nutrient delivery (Chen et al., 2008). Based on Table 2.1, it shows the design criteria for bone tissue engineering scaffolds.

Table 2.1: Design criteria for bone tissue engineering scaffolds (Chen et al., 2008)

Criteria	Description
Biodegradability	Combination between the composition of the material with the porous structure of the scaffold should results in biodegradation at rates suitable to tissue regeneration.
Mechanical properties	The mechanical strength of the scaffold which is affected by both the properties of porous structure and the biomaterial should be enough to give mechanical stability to constructs in load bearing sites before synthesis of new extracellular matrix by cells.
Fabrication	The material should possess desired fabrication capability, e.g., being readily produced into irregular shapes of scaffolds that match the defects in bone of individual patients.
Ability to deliver cells	The material should not only be biocompatible, but also encourage the cell attachment, proliferation, and differentiation.
Osteoconductivity	Osteoconductivity does not only remove the formation of fibrous tissue encapsulation but it also produce strong bond between the host bone and scaffold.
Porous structure	The scaffold should have an interconnected porous structure with porosity > 90% and diameters between 300-500 μm for

	cell penetration, tissue ingrowth and vascularization, and nutrient delivery.
Commercialization potential	The synthesis of the material and fabrication of the scaffold should be suitable for commercialization.

Biomaterials are designed to encourage the growth, organization, and differentiation of cells in the process of producing functional tissue by providing structural support, chemical clues, and biological containment. In biomaterial research, fabrication and scaffold design are major areas, and they are also important point for tissue engineering and regenerative medicine research. Therefore, advance scaffolds that have the optimal characteristics such as their porosity, rate of degradation, strength, and microstructure, also as their sizes and shapes, are more suitable and reproducibly controlled in polymeric scaffolds (Dhandayuthapani et al. 2011).

2.2.1 Polymer

Some reviews have explained several kinds of degradable polymers and their co-polymers. The features of a degradable polymer to be respected prior to implantation have been divided into two main categories which is biofunctionality and biocompatibility. Biofunctionality refers to the characteristics of adequate properties (physical, mechanical, thermal, chemical and biological), easy to handle, sterilizable, resorbable and storable. While, biocompatibility refers to the aspects concerning the absence of toxicity, carcinogenicity, immunogenicity, and thrombogenicity. Nowadays, natural and synthetic polymer have been widely used in many medical devices and tissue engineering

applications. Table 2.2 shows some example of the polymers that used in tissue engineering and biomedical devices.

Table 2.2: Some of the polymers that used in tissue engineering and biomedical devices (Saltzman, 2004).

Materials	**Typical Applications**
Collagen	Artificial skin
Polyamides	Dialysis membranes Sutures
Poly(ethylene terephthalate) (PET)	Vascular grafts Artificial hearts
Poly(L-lactic acid), Poly(glycolic acid) and Poly(lactide-co-glycolide) (PLA, PGA and PLGA)	Drug delivery vehicles
Poly(methyl methacrylate) (PMMA)	Bone cement for fracture fixation Dentures
Poly(tetrafluoroethylene) (PTFE)	Heart valves Vascular grafts Membrane oxygenators Catheters and sutures
Polyethylene (PE)	Hip prostheses
Polyurethanes(PU)	Catheters Pacemaker leads Artificial hearts and ventricular assists devices
Polydimethylsiloxane (PDMS)	Drug delivery vehicles Hearts valves Catheters

Biodegradable polymers have transformed the uses of biomaterial in the field of drug delivery and implants for tissue engineering applications (Dhandayuthapani et al. 2011). Degradation of scaffold can occur by mechanisms that took part in physical or chemical processes and/or biological processes that are facilitated by biological agents, such as enzymes in tissue remodelling. Degradation could also results in scaffold disassembling and material dissolution/resorption through the scaffolds bulk and/or

surface types of degradation. Hydrolysis process will result in reduction of the polymer's molecular weight which will decrease the strength of implant material.

2.2.1.1 Natural polymers

In tissue engineering, there are two types of natural polymers that is commonly used which is proteins and polysaccharides. For proteins categories, the example of natural polymers is fibrin, collagens and gelatine. While for polysaccharides, it involves hyaluronic acid (HAc), chitosan, chitin, and alginate. Most of the naturally occurring polymers are extracted from animals or plants. In addition, the natural polymers that widely applied in tissue engineering are HAc and collagen as they will encourage the cells growth and attachments (Harrison 2007). Natural polymer also supply a natural substrate for cellular proliferation, attachment, and differentiation and are considered preferred substrates for tissue engineering.

Besides, they are normally biodegradable (via hydrolysis), highly biocompatible, and thermos-processable, thus being attractive for application in tissue engineering (Chen et al., 2008). They function as intrinsic templates for cell growth and attachment also they could encourage an immune response at the same time. In addition, the advantage of natural polymer materials is that the structures is highly organized and it contains additional cellular substance which is known as a ligand that can be bound to cell receptors. However, there are some concerns regarding immunogenic problems associated for example with the introduction of foreign collagen (Vacanti et al., 2007). The saccharide units that are present in each different bio-polymers are shown in Figure 2.2.

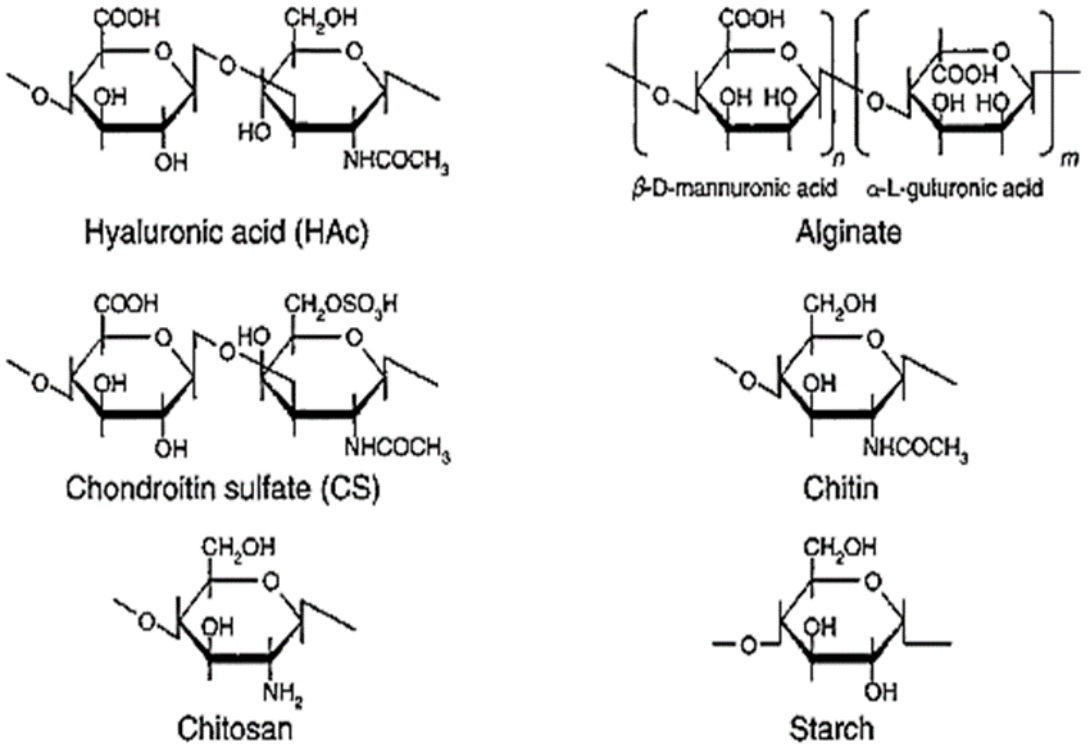

Figure 2.2 Molecular structure of saccharides units that presence in the backbone of each biopolymers (Ikada, 2006).

There are some disadvantages of natural polymer materials even though they are known as biocompatible such as deficiency in expansive, bulk quantity, and difficulties in the processability for scaffold in clinical applications (Sabir et al. 2009). From patient to patient, there are some differences in the degradation rate of natural polymer materials because the degradation of natural polymer materials depends on the enzyme which is not the same from one patient to another patient. Polyhydroxybutyrate (PHB) is of particular interest for bone tissue engineering considering that a consistent favorable bone tissue adaptation response was demonstrated with no evidence of undesirable chronic inflammatory response after implantation periods up to 12 months (Misra et al., 2006).

Collagen, aid in the regeneration of tissues, commonly for the repair of soft tissues. Collagen targets the cell adhesion and offers cellular recognition for control cell attachment and performance and it might guide to the priority of unfavorable reaction. According to Sabir et al. (2009), collagen undergoes catalyst degradation that happens in body via enzymes, like collagenases and metallo-proteinases, to yield the corresponding amino acids. Because of their catalyst degradation, distinctive physico-chemical, mechanical, and biological properties area unit studied in numerous applications. The composite of collagen and hydroxyapatite and TCP (tri calcium phosphate) is applied to biodegradable synthetic bone graft replacement (Sabir et al. 2009).

Polysaccharides area unit macromolecules made from an oversized variety of monosaccharose units joined along by gly-cosidic linkages and are naturally derived polymers have usually utilised in biomaterials field. Rising of recognition on the potential of saccharide as biomaterials are contributed by three completely different aspects (Suh and Matthew, 2000). It has the distinctive property of cell signals to immune. Besides, the biodegradability and talent to fabricate applicable structures making them one among the foremost necessary and wide studied biomaterials.

Chitosan is a cationic linear polysaccharide containing of b (1-4) linked D-glucosamine with randomly located N-acetylglucosamine groups depending upon the degree of deacetylation of the polymer (Sabir et al. 2009). Depending on the degree of polymer crystallinity, the degradation rate of chitosan is different as it was degraded by lysozyme. Thus, the degradation rate of a chitosan will reduce as the crystallinity degree increases.

They are block copolymers composed of regions of sequential (1-4)-linked β-$_D$-mannuronic acid monomers (M-blocks) and α-$_L$-guluronic acid (G-blocks) with interspersed region of M and G blocks (Ikada 2006). The high practicality of alginic acid

makes it a biocompatible material. While, it is widely used as cell transplantation vehicles to grow new tissues also as wound dressing. The drawbacks of those compound materials are slow degradation and not enough of mechanical integrity that make it not possible for future implants.

2.2.1.2 Synthetic polymers

Synthetic polymers are usually cheaper than life scaffolds and it is created in giant uniform quantities while having an extended shelf time. These polymers are specifically considered in tissue engineering strategies because of their potential ability to encourage cell adhesion, migration, proliferation and differentiation (Ryszkowska et al., 2010). Synthetic polymers are preferable for clinical and surgical applications because it is feasible to regulate their mechanical properties and degradation rates betting on the actual application. After the occurs of Creutsfeld-Jacobs disease, the focus of biomaterials scientists have transform from natural polymers into non-biological materials, such as synthetic polymers (Ikada 2006).

It have some advantages, such as outstanding processing characteristics, which can ensure the off-the-shelf availability as well as being biocompatible and biodegradable at rates that can be tailored for the intended application (Chen et al., 2008). There are two types of synthetic polymer which is bulk biodegradable and surface bioerodible polymers, the previous have shown additional promise considering that one of the requirements of a tissue engineering scaffold is that it's to get replaced by recently shaped bone tissue in vivo.

Several commercially on the market artificial polymers show physicochemical and mechanical properties compare to those of biological tissues. Synthetic polymers

represent the most important cluster of biodegradable polymers, and that they is created underneath controlled conditions. But, scaffolds made of artificial polymers do not exhibit adequate mechanical properties and bioactive behaviour that may be a disadvantage for bone tissue engineering applications.

Biodegradable synthetic polymer materials such as poly (glycolic acid), poly (lactic acid), and their copolymers, poly (p-dioxanone), and copolymers of trimethylene carbonate and glycolide plays an important role in clinical applications (Sabir et al. 2009). There are two types in biomaterials area which is synthetic polymers absorbable and non-absorbable (Ikada 2006). Absorbable polymers are used as key materials for the bone tissue engineering applications as they function temporary template and deteriorate when the tissue had totally remodelled. While, non-absorbable polymers have been used as main materials to be used in artificial organs, implants, and other medical devices.

2.2.1.3 Polyurethane scaffold

Polyurethanes (PU) are engaging candidates for scaffold fabrication, since they are biocompatible, and have very good mechanical properties and mechanical flexibility. Biodegradable polyurethanes, which produced from degradable polyester/polyether with hydrophilic group of ether bond, aliphatic diisocyanate, contain the hydrophobic group of alkly and chain extenders (Huang et al. 2009). Besides, they have distinctive divided structure, due to which more numerous properties may be obtained victimization relevant raw materials and additives. According to Janik & Marzec (2015), PU will have a difference in mechanical and physical properties, from thermoplastic to thermoset, from stable to degradable materials, from hydrophobic to hydrophilic depending on the composition and synthesis procedure applied.

PU is an organic polymer consist of organic units (urethane group) in the polymer structure. It is typically produced between the reaction of polyol and diisocyanate. It is formed on aromatic isocyanates characterised by a lack of biocompatibility due to the toxic degradation products released from the aromatic hard segment. The chains of the PUs can be enlarged by silane groups by the reaction of the prepolymer with aminosilanes. Figure 2.3 shows chemical structure of a polyurethane.

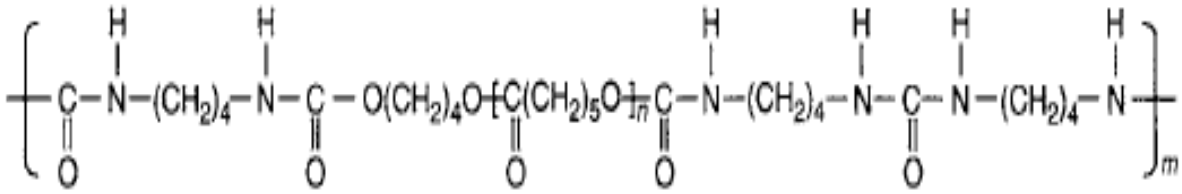

Figure 2.3: Chemical structure of Polyurethane (PU) (Ikada, 2006)

The polymer had given a name known as polyurethane due to the existence of urethane linkage in polymeric. Biodegradable polyurethanes, made up from degradable polyester/polyether with hydrophilic group of ether bond, aliphatic diisocynate, having the hydrophobic group of alkly and chain extenders (Guelcher et al., 2005). The existence of special groups causing the polyurethanes function in controlling the degradation rate.

PU shows elastic properties, thermo-plasticity and durability. According to Bil et al. (2009), these properties makes them very suitable to be use in various of tissue engineering either for rebuilding of soft tissue for cartilage and bone regeneration. Afterwards, their designed was suitable to be used in biomedical applications such as soft tissue engineering, cardiac tissues, materials for use in contact with the blood, implants for the meniscus knee, and controlled release of drugs. In addition, PU consists of hard segment and soft segment that combine together to give the PU matrix moderate stiffness,

high rubber-like extensibility, easy processability and good biocompatibility (Liff et al. 2007).

For tissue engineering, one of the important factors in selecting the materials is the degradation kinetics. Deterioration of polyurethane occurs when reaction with the water take part and the reaction rate can be improved either in alkaline or acidic environment (Clemitson 2008). Hence, the kinetic of degradation for polyurethane must be carefully controlled when it serves in human body. As bone repair material, PU or PU scaffold loaded with nano-hydroxyapatite (n-HA) particles could increase its bone-bonding bioactivity and mechanical properties (Bil et al., 2008). Moreover, the degradation give rise to non-toxic products, which will not causing side effect for body. Chain extender is also a key factor besides polyester/polyether and diisocyanate.

2.2.2 Bioactive glass

The name ‘‘Bioglass’’ was trademarked by the University of Florida as a name for the original 45S5 composition (Jones 2013). According to Hench, (2006), the composition of glass with 45% SiO_2, 24.5% Na_2O, 24.5% CaO, and 6% P_2O_5 was chosen to provide a large amount of CaO with some P_2O_5 in a Na_2O-SiO_2 matrix which these composition is very near to a ternary eutectic, making it easy to melt. Bioactive glasses, such as standard 45S5 Bioglass (BG), are being widely taken in the areas of bone tissue engineering.

Afterwards, bioglass was the earliest synthetic material found to form a chemical bond with bone, launching the field of bioactive ceramics or which known as bioceramics (Ong et al., 2015). Usually bioactive glasses have been used to fill and restore bone defects. It is applied as bone graft materials for small bone defects as bioactive coatings

for orthopaedic implants and as filler particles in biopolymer composites. During implantation of bioactive glass in the body, they stimulate an interfacial bioactive behaviour (Kokubo, 2005). According to Narayan et al. (2012), later it shows that this glass is better integrated into the bone when the structure is porous. The in vitro tests revealed that the 45S5 Bioglass composition developed a hydroxyapatite layer in test solutions that did not consist of calcium or phosphate ions.

There are various types of bioactive glass which is the conventional silicates, such as Bioglass 45S5, phosphate-based glasses, and borate-based glasses. In addition, calcium phosphates such as tricalcium phosphate and synthetic hydroxyapatite also widely applied in the clinic. Lately, interest has increased in borate glasses, mostly due to very encouraging clinical results of healing of chronic injuries, such as diabetic ulcers, that would not heal under conventional treatment (Jones 2015) . The soft tissue reaction may be due to their fast dissolution, which is more rapid than that for silica-based glasses. The benefits of phosphate glasses are also probable to be interrelated to their very rapid solubility rather than bioactivity.

In general, with the composite scaffolds composed of bioactive glasses and PCL-based materials (regardless of method of fabrication and glass size), porosity somewhat decreased with glass content and pore shapes were uneven. However, in most cases, it shows 1–5% reduction in porosity. Other studies have investigated the effect of bioactive glass coats on the mechanical, degradative and bioactive properties of PU foams. The scaffold made up from PU was coated by SiO_2–P_2O_5–CaO–MgO–Na_2O–K_2O bioactive glass using a slurry coating method (Zeimaran et al. 2015). The stiffness and strength of the scaffold were greater than that of the uncoated.

Manufacturing techniques for bioactive glasses include both traditional melting methods and soil-gel techniques. According to Gerhardt & Boccaccini (2010), the typical

feature common to all bioactive glasses, being melted or sol-gel derived, is the ability to interact with living tissue, in particular forming strong attachments to bone (and in some cases soft tissue, a property commonly termed bioreactivity or bioactivity, as noted supra. Nowadays, it is accepted that for establishing bonds with bone, such a biologically active apatite surface layer must format the material/bone interface.

Then, bone bonding involves in formation of HCA layer, which interacts with collagen fibrils of damaged bone to form a bond (Gerhardt et al., 2007). HCA is same with bone mineral and interact with collagen fibrils to integrate (bond) with the host bone. The properties of osteogenic of the glass are thought to be due to the dissolution products of the glass, i.e. soluble silica and calcium ions, that stimulate osteogenic cells to produce bone matrix.

Some of the reasons are commercial, but others are due to the scientific limitations of the original Bioglass 45S5. An example is that it is hard to produce porous bioactive glass templates (scaffolds) for bone regeneration from Bioglass 45S5 because it crystallizes during sintering. Besides, bioceramics are of the biomaterials that commonly used in bone tissue engineering, other than biopolymers (Chatzistavrou et al. 2011). It consist of weak mechanical strength and low toughness of bioactive glasses which has prevented their use as load bearing devices (Hench, 2006).

Bioactive glass shows excellent compressive strength (Vallet-Regí 2014). In vivo studies have shown that bioactive glasses bond with bone more quickly than other bioceramics, and in vitro studies show that their osteogenic properties are due to their dissolution products stimulating osteoprogenitor cells at the familial level (Jones 2013). Table 2.3 shows the mechanical properties of human trabeculan, corticular bone and dense Bioglass 45S5.

Table 2.3: Mechanical porperties of human trabecular, cortical bone and dense Bioglass® 45S5 (Gerhardt and Boccaccini, 2010)

Mechanical properties	**Trabecular bone**	**Cortical bone**	**Bioglass® 45S5**
Compressive strength (MPa)	0.1 – 1.6	130 – 200	500
Tensile strength (MPa)	n.a.	50 – 151	42
Compressive modulus (GPa)	0.12 – 1.1	11.5 – 17	n.a.
Young's modulus (GPa)	0.05 – 0.5	7 – 30	35
Fracture toughness ($MPa.m^{1/2}$)	n.a.	2 – 12	0.7 – 1.1

Table 2.3 shows that the compressive strength of bioactive glass scaffolds is more than 1.5 times greater than the highest strength reported for trabecular bone with range between 0.1–16 MPa. In addition, bioactive glass has a better adhesion to stainless steel due to its high thermal expansion and bioactivity (Ong et al., 2015). Addition of bioglass to agarose scaffolds improve the biochemical and mechanical properties of the tissue-engineered cartilage layer. According to Ong et al. (2015), when BG is implanted into rabbits, it results in thicker cartilage-like tissue and at the same time improve the biomechanical properties with more cartilage matrix content than constructs without bioglass.

2.3 Composites scaffold

Composites developed by a combination of bioactive glass and biodegradable polymers are attractive materials to be used as scaffold by musculoskeletal tissue engineering because of their ability to be tailored for different application (Verrier et al., 2011). In order to encourage and guide new bone formation scaffold must be osteoconductive and osteoinductive. From the materials science point of view, a single material type does not usually provide the necessary mechanical and/or chemical properties required, hence the properties of two or more materials can be blended in a composite textile. The dispersed phase presence in the composite materials or scaffolds is considered as a reinforcement component as they are stronger than the matrix form.

From a biological view, it builds sense to combine polymers and bioceramics to fabricate scaffolds for bone tissue engineering because native bone is the combination of a naturally occurring polymer and biological apatite (Chen et al., 2008). Mechanically, glasses and bioceramics are stronger than polymers and play a critical role in providing mechanical stability to constructs prior to synthesis of new bone matrix by cells. However, ceramics and glasses are very fragile and prone to catastrophic failure due to their intrinsic brittleness and flaw sensitivity.

In order to satisfy as many demands as possible, composite scaffolds may be an interesting solution. Polymer-ceramic composite scaffolds, in fact, may combine the toughness, reliability and slow-rate degradation of the polymer phase with the highest bioactivity of bioglasses, hydroxyapatite and other ceramic phase. Therefore, it cannot provide the essential mechanical needs required in bone tissue engineering application (Verrier et al. 2011).

Combination of both materials (biodegradable polymers with bioactive glass) is anticipated to increase the strength of polymeric matrix due to the inclusion of bioactive glass in the polymeric matrix (Ikada 2006). Also, with the advantage of the flexibility and formability of PU and controlled volume fraction of BG as reinforcement, fabrication of scaffold with suitable mechanical reinforcement to an average stress of approximately 4 MPa for daily activities can be achieved (Ramakrishna et al. 2001). Figure 2.4 shows the schematic diagram of composite scaffolds fabrication process.

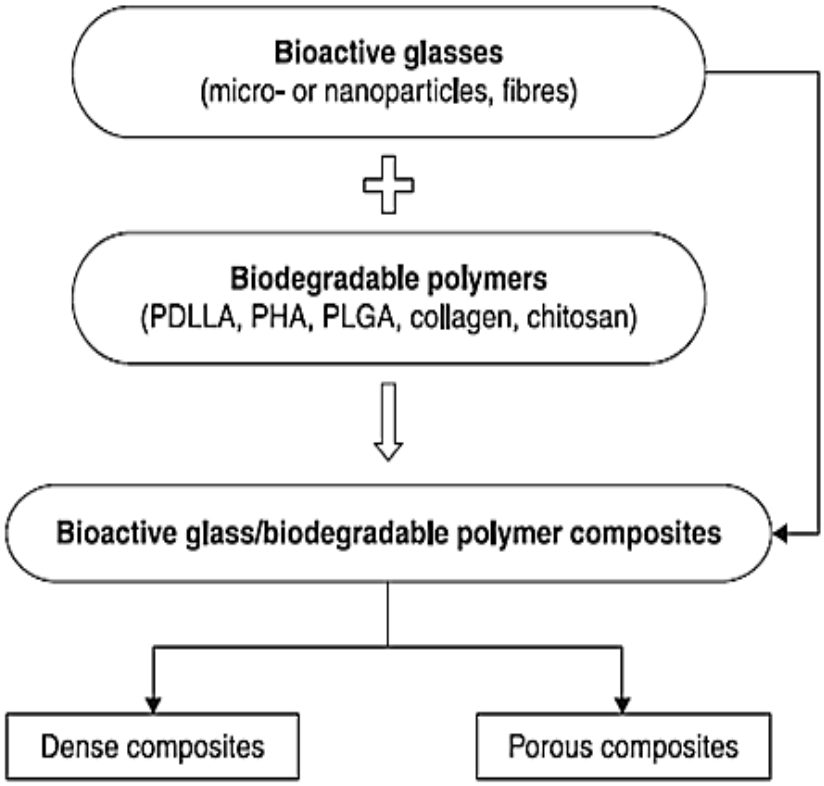

Figure 2.4: Schematic diagram of bioactive glass/polymer composite scaffolds fabrication process (Verrier et al. 2011).

One of the requirements imposed on scaffolds is a suitable, porous structure with uniformly distributed interconnected pores. Materials should be characterised by great porosity (above 90%) and proper pore dimension (from ten to hundreds of μm) depending