PROPERTIES OF BIOACTIVE GLASS REINFORCED POLYURETHANE SCAFFOLD BY SOLVENT CASTING/PARTICULATE LEACHING METHOD

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by

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LIST OF SYMBOLS

mm	milimeter
wt%	Weight percentage
%	Percentage
wt/vol	Weight per volume
µm	Micrometer
°C	Degree celcius
β	Beta
cm^{-1}	Per centimeter
M	Molar
MPa	Mega Pascal
GPa	Giga Pascal
g	Gram
mg	Milligram
g/cm^3	Gram per cubic centimeter
rpm	Rotation per minute
V_s	Volume of Hank's Balanced Salt Solution
S_a	Surface area of the specimen
mm^2	Square millimeter
mm/min	Millimeter per minute
λ	Lamda
kV	Kilo Volt
mA	Milli Amphere
°/min	Degree per minute

T_g	Glass transition temperature
T_m	Melting temperature
Π	Percentage of porosity
$\rho_{scaffold}$	Bulk density of the scaffold
$\rho_{material}$	True density of the material
kN	Kilo Newton
wt/vol %	Percentage of concentration in weight per volume
Ti	Titanium
Zn	Zinc
Mg	Magnesium
Sr	Strontium
B	Boron
Al	Aluminium
O	Oxygen
P	Phosphorus
Si	Silicon
Na	Sodium
Ca	Calcium
Mn	Manganese

LIST OF ABBREVIATIONS

1,4-BDI	1,4-butane diisocyanate
1,6-HDI	1,6-hexamethylene diisocyanate
3D	Three-dimensional
A/W	Apatite-wollastonite
Al_2O_3	Alumina
ASTM	American Society for Testing and Materials
ATR	Attenuated total reflectance
BD	Butanediol
$CaCO_3$	Calcium carbonate
CaO	Calcium oxide
Ca-P	Calcium-phosphate
COOH	Carboxylic
dHEBA	α,ω-dihydroxy(ethylene-butylene adipate)
DMSO	Dimethylsulfoxide
DPS	Degree of phase separation
DSC	Differential scanning calorimetry
DTG	Derivative thermogravimetric analysis
EDX	Energy dispersive X-ray
FDA	United States Food and Drug Administration
FESEM	Field emission scanning electrons microscopy
FTIR	Fourier transform infrared spectroscopy
H_2O_2	Hydrogen peroxide
HA	Hydroxyapatite

HBSS	Hank’s Balanced Salt Solution
HCA	Hydroxycarbonate apatite
HCl	Hydrochloric acid
HMDI	4,4’-dicyclohexamethylene diisocyanate
ICP	Inductively coupled plasma
IPDI	Isophorone diisocyanate
KBr	Potassium bromide
KCl	Potassium chloride
LDI	L-Lysine methyl ester diisocyanate
Lys	Lysine ethyl ester
MDI	4,4’-diphenylmethane diisocyanate
MSCs	Mesenchymal stem cells
N_2	Nitrogen gas
Na_2CO_3	Sodium carbonate
Na_2O	Sodium oxide
Na_2SO_4	Sodium sulfate
NaCl	Sodium chloride
$NaHCO_3$	Sodium hydrogen carbonate
NaOH	Sodium hydroxide
nFHA	Nano-flour hydroxyapatite
NH_4HCO_3	Ammonium hydrogen carbonate
OH	Hydroxide
P_2O_5	Phosphorus pentoxide
PBS	Phosphate buffered saline
PCL	Polycaprolactone

PCTC	Polycaprolactone-block-polytetrahydrofuran-block-polycaprolactone
PDLLA	Poly(D,L-lactide)
PEEUU	Poly(ether ester urethane)urea
PGA	Poly(glycolic acid)
PLA	Poly(lactic acid)
PLGA	Poly(DL-lactic-co-glycolic acid)
POC	Poly(octanediol citrate)
PSA	Particle size analyzer
Pt-Au	Platinum-Aurum
PU	Polyurethane
PU-BG	Polyurethane-Bioglass®
SBF	Simulated body fluid
SC/PL	Solvent casting/particulate leaching
SiO_2	Silicon dioxide
TDI	Toluene diisocyanate
TGA	Thermogravimetric analysis
THF	Tetrahydrofuran
TIPS	Thermal induced phase separation
UTM	Universal testing machine
VMD	Volume mean diameter
WA	Water absorption
WL	Weight loss
WO	Tunsten oxide
XRD	X-ray diffraction

XRF	X-ray flourescence
B-TCP	Beta-tricalcium phosphate

SIFAT-SIFAT PERANCAH POLIURETANA DIPERKUAT KACA BIOAKTIF DENGAN KAEDAH TUANGAN PELARUT/PARTIKEL LARUT LESAP

ABSTRAK

Sifat-sifat perancah poliuretana-Bioglass® (PU-BG) telah dioptimumkan menggunakan kaedah tuangan pelarut/partikel larut lesap (SC/PL). Parameter pemprosesan seperti kepekatan larutan polimer (15, 16, 17, 18 dan 19 wt/vol%), kepekatan agen pelarut dengan nisbah antara PU dan NaCl (1:3, 1:4, 1:5 1:6 dan 1:7), komposisi agen pelarut melalui peratusan NaCl/$NaHCO_3$ (100/0, 85/15, 75/25, 65/35, 55/45 dan 45/55) dan peratusan Bioglass® (0, 10, 20, 30, 40 dan 50 wt%) telah dikaji bagi mendapatkan ciri-ciri perancah PU-BG yang dikehendaki. Perancah tersebut telah diuji menggunakan teknik mikroskopi imbasan elektron (FESEM), ujian keliangan, analisis infra-merah (FTIR), ujian mampatan, plasma aruhan gandingan (ICP) dan ujian biodegradasi. Parameter optimum untuk fabrikasi perancah PU-BG yang telah didapati ialah 19 wt/vol % bagi kepekatan larutan polimer, nisbah 1:6 bagi kepekatan agen pelarut dan 65% NaCl dan 35% $NaHCO_3$ bagi komposisi agen pelarut. Proses pengoptimuman telah berupaya menghasilkan perancah komposit dengan ketebalan melebihi daripada 20 mm tanpa pembentukan lapisan padat yang tidak diingini. Disamping itu, perancah PU-BG diperhatikan dengan saiz liang yang merangkumi julat yang luas dan peratusan keliangan yang mencukupi untuk aplikasi tisu tulang. Keputusan menunjukkan, peningkatan dalam peratusan Bioglass® dari 0 wt% ke 50 wt% telah didapati memperbaiki kekuatan mampat perancah PU-BG dan juga meningkatkan dan mempercepatkan pembentukan apatit ketika ujian in-vitro.

PROPERTIES OF BIOACTIVE GLASS REINFORCED POLYURETHANE SCAFFOLD BY SOLVENT CASTING/PARTICULATE LEACHING METHOD

ABSTRACT

The properties of polyurethane-Bioglass® (PU-BG) scaffolds have been optimized using solvent casting/particulate leaching (SC/PL) method. Processing parameters such as polymer solution concentration (15, 16, 17, 18 and 19 wt/vol%), leaching agent concentration with ratio between PU and NaCl (1:3, 1:4, 1:5, 1:6 and 1:7), leaching agent composition by percentage of NaCl/$NaHCO_3$ (100/0, 85/15, 75/25, 65/35, 55/45 and 45/55) and percentage of Bioglass® (0, 10, 20, 30 40 and 50 wt%) were investigated to obtain the desired properties of PU-BG scaffolds. The scaffolds were characterized using field emission scanning electron microscopic (FESEM), porosity test, fourier transform infrared (FTIR), compression test, inductively coupled plasma (ICP) and degradation test. The optimum parameters to fabricate PU-BG scaffold were found to be 19 wt/vol % of polymer solution concentration, 1:6 ratio (PU: NaCl) of leaching agent concentration and leaching agent composition between 65% NaCl and 35% $NaHCO_3$. The optimization process was able to fabricate the composite scaffold with the thickness more than 20 mm without the formation of undesirable dense layer. Besides, the PU-BG scaffolds were observed with well interconnected porous structure with the pore size spanning over a wide range and adequate porosity for the bone tissue application. The results demonstrated that, the increased in percentage of Bioglass® from 0 wt% to 50 wt% was found to improve the compressive strength as well as induced and accelerated an apatite formation during in-vitro test.

CHAPTER ONE

INTRODUCTION

1.1 Background of the research

Tissue engineering is an emerging multidisciplinary and interdisciplinary field consisting the development of bioartificial implants and/or the nurturing of tissue remodelling with the objective to repair and enhance tissue or organ function (Nerem and Sambanis, 1995). In 2016, Global Industry Analyst Inc, launched research on global industry found that the global market for Bone Graft Substitutes is projected to reach US$3.2 billion by 2022. This high demand for bone graft substitutes is driven by a growing number of orthopaedic surgeries performed worldwide and the growth of novel bone graft substitutes and materials. Three-dimensional (3D) scaffold is the main application in tissue engineering which provides a suitable microenvironment for the incorporation of cells or growth factors to regenerate damaged tissues or organs. Biocompatible, biodegradable and able to promote cellular interactions and tissue development as well as possess proper mechanical and physical properties are the requirements for ideal scaffold (Liu and Ma, 2004).

Biomaterial is defined as nonviable material used in medical device, intended to interact with the biological system (Ratner *et al.*, 2006). Biomaterial covers both synthetic and natural materials which can be implanted in the human body for the purpose of health improvement. The wide variation of biomaterials have been developed for bone tissue engineering application comprises from naturally derived materials to synthetic biopolymers (Kim *et al*., 2009).

For decades, synthetic biodegradable polymers had offered a number of advantages for the developing of scaffold in tissue engineering. The tailorable

properties (e.g., porosity, degradation time and mechanical characteristic) of the synthetic polymer depending specific application is useful in the biomedical field. The ability of the synthetic polymer to be fabricated into various shapes with desired pore morphologies features is also conducive for tissue growth. Over the decades, polyurethane (PU) has been extensively studied as potential polymer in biomedical application due to its exceptional biocompatibility, tailorable mechanical properties and versatility. Biodegradable PU has proved its ability to support the ingrowth of cells with controllable degradation to non-cytotoxic decomposition products (Patel *et al.*, 2011). However, the lack of bioactive properties limits the application of the PU. Hence, one of the solutions is to incorporate PU with bioactive glass such as Bioglass® 45S5. Bioglass® 45S5 is belong to SiO_2-Na_2O-CaO-P_2O_5 system was first discovered by Hench in 1969-1671 (Hench, 2006). The dissolution products of the glass stimulates progenitor cells to differentiate a bone cell (osteoblast) pathway by stimulating genes associated with osteoblast differentiation (Hench and Polak, 2002; O'Donnell, 2012). Hence, Bioglass® is considered as "class A bioactive materials" as it is able to make a strong bond for both hard and soft tissues in vivo.

The focus of tissue engineering is the fabrication of scaffold as a functional replacement for damaged tissue or organs. Numbers of methods had been applied in the fabrication of polymer scaffold including thermal induced phase separation (TIPS), gas foaming, melt moulding and solvent casting/particulate leaching (SC/PL). Among the methods, SC/PL or known as salt leaching method is one of the versatile methods to fabricate polymer scaffold. Pore size and porosity of the scaffold can be tailored by using leaching agent particles with specific size and concentration for the targeting cell type. Besides, SC/PL technique does not imply any high temperature that preserve the properties of polymer. Fabrication of polymer scaffold by SC/PL is prepared by

dispersing salt particles in polymer solution followed by elimination of solvent. The pore structure is created by leaching out the salt particles in water or other suitable solvents for dissolving the salt. By utilizing SC/PL technique, few factors should be considered for instance polymer solution concentration (Bil *et al.*, 2009), leaching agent concentration (Reignier and Huneault, 2006), size of leaching agent and type of leaching agent (Cannillo *et al.*, 2010). All these aspects will influence the pore structure, interconnectivity, porosity, mechanical properties and other properties required by the scaffold.

1.2 Problem statement

Bioactive glass is often used as a reinforcing agent that combined with polymers in order to imitate the combination of mineral bone and collagen of natural bone composite. Several studies had demonstrated incorporation of bioactive glass with biodegradable polymer able to improve the bioactivity and osteoconductivity of the bioactive glass reinforced polymer scaffold (Maji and Dasgupta, 2015; Murphy *et al.*, 2017; Liverani *et al.*, 2018). In another point of view, PU is one of the biodegradable polymers which has drawn attraction in tissue engineering due to its segmented structure and tailorable properties. Many studies had investigated the potential of PU scaffold for bone tissue application (Kucinska-Lipka *et al.*, 2017; Aguilar-Perez *et al.*, 2018; Meskinfam *et al.*, 2018). Despite of the advantages offered from both PU and bioactive glass, however only few studies had focused on the development of bioactive glass reinforced PU scaffold for bone tissue regeneration (Ryszkowska *et al.*, 2010; De Oliveira *et al.*, 2012; Hafezi *et al.*, 2016). Based on the fabrication of PU scaffold from the previous study (Ryszkowska *et al.*, 2010), SC/PL technique is the most versatile technique which gave a promising porous structure as

required for bone tissue engineering. However, there are lack of information on the processing parameters that influence the properties of bioactive glass reinforced PU scaffold by SC/PL technique.

SC/PL is a well-known technique in polymeric scaffold fabrication due to its versatility and ease of fabrication. The process involves the dispersion of the leaching agent into a polymer solution followed by casting, drying and leaching process. The porous structure of the scaffolds can be easily controlled by the concentration and size of the leaching agent (Bil *et al*., 2009). However, scaffold prepared by SC/PL is only limited to very thin wall sections approximately from 0.5 until 2.0 mm in thickness. As a consequence, scaffold with a thin wall is limited only for a non-load bearing tissue application such as blood vessel graft, nerve regeneration and wound healing. Besides that, SC/PL technique is constrained by the formation of dense layer on the top layer of the scaffold. The formation of dense layer on the scaffold is undesirable as it will hampered in-vivo cell seeding into the scaffold and ingrowth of tissue through the scaffold (Nam *et al*., 2000). Due to these limitations involving SC/PL, only few studies dedicated their work to fabricate PU scaffold by this technique which focusing on bone tissue regeneration (Bil *et al.*, 2009; Ryszkowska *et al.*, 2010; Asefnejad *et al.*, 2011a; Wosek, 2015).

Studies investigated by Bil et al., (2009) and Ryszkowska *et al.*, (2010) which fabricated PU scaffolds by SC/PL using 1:5 ratio of polymer to leaching agent resulted in different range of porosity. Both studies recorded the porosity within the range of 78-82 % and 67-88%. Meanwhile, another study conducted by Asefnejad *et al.*, (2011a) also fabricated PU scaffolds using 1:2 ratio recorded only with a slight decrease in porosity (71-75 %). Although the mentioned studies used different concentration of leaching agent to fabricate PU scaffold, the porosity of the scaffold

does not have very large deviations with each other. Hence, there is inconsistent guidelines can be referred for the future studies to fabricate PU scaffold by SC/PL technique.

In response to these problems, this study proposed to prepare a basic complete process design for the fabrication of PU scaffold. Several parameters such as polymer solution concentration, leaching agent concentration and leaching agent composition which influence the properties of the PU scaffold need to be investigated and optimized. Influence of Bioglass® percentage on the properties of the scaffold also need to be studied as it could affect the bioactivity and degradation behaviour of the PU scaffold. Besides investigating the optimum process parameter for the fabrication of PU scaffold, this study also aims to eliminate the disadvantages regarding the thin wall section and dense layer formation associated with SC/PL technique. By optimizing the SC/PL technique, a complete set of data on the properties of the PU scaffold fabricated by this technique will be prepared which could be useful as a reference for future studies to fabricate PU scaffold for future improvements.

1.3 Objectives

The main objective of this study is to optimize the properties of PU reinforced Bioglass® scaffolds by SC/PL method in order to improve the scaffold's performance. To achieve the main target, the specific objectives in scaffold's optimization by SC/PL technique are described as follows:

i. To identify the influence of polymer solution concentration, leaching agent concentration, leaching agent composition and percentage of Bioglass® on the morphology, physical, chemical and mechanical properties of PU-Bioglass® (PU-BG) scaffolds.

ii. To determine the effect of Bioglass® on the bioactivity and degradation behaviour of PU-BG scaffolds.

1.4 Thesis outline

Chapter 1 starts with a research background by briefly introduce about tissue engineering and overview of the study. This chapter also describes the problem statements in the fabrication of polymeric scaffolds by SC/PL technique aiding with the objectives of this project.

Chapter 2 describes the literature review based on other studies. It starts with the introduction of tissue engineering with the main objective is to develop a scaffold conforming to the requirement. Potential biomaterials and fabrication techniques for scaffolds are also explained in this chapter.

Chapter 3 illustrates the experimental procedure for PU and Bioglass® synthesize as well as PU-BG scaffolds fabrication. A flow chart is provided to guide as the fabrication of PU-BG scaffolds is divided into four stages of optimization: Stage 1 (polymer solution concentration), Stage 2 (leaching agent concentration), Stage 3 (leaching agent composition) and Stage 4 (Bioglass® percentage). Besides, the characterization methods are also apecified in this chapter.

Chapter 4 is divided into a few parts started with the characterization report of the synthesized PU and Bioglass® to confirm the obtained materials following the requirement. The properties of the fabricated PU-BG scaffolds is discussed in each stage of optimization. The effect of polymer solution concentration, leaching agent concentration, leaching agent composition and percentage of Bioglass® on the scaffolds are elaborated in term of morphology, physical, functional group and mechanical properties of the composite scaffolds. At the end of each stage, the optimum parameter for the scaffold's fabrication by the SC/PL technique is finalized

based on the properties of the PU-BG scaffolds. In-vitro bioactivity and degradation of the PU-BG scaffolds are evaluated based on the Bioglass® percentage in the scaffold at the end of this chapter.

Chapter 5 presents the conclusion from this project based on the properties assessment of the PU-BG scaffolds. The optimum parameter for SC/PL technique of PU-BG scaffold's fabrication is finalized.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Scaffolds fabrication is the main focus in tissue engineering as it plays a unique role in bone tissue regeneration and repair (Dhandayuthapani *et al.*, 2011). In past few decades, lots of studies working on designing a scaffold with highly porous and well-interconnection structure, biocompatible and bioresorbable materials, excellent bioactivity and mechanical properties mimic with the natural bone (Cannillo *et al.*, 2010). Henceforward, PU is one of the biomaterials that has attracted great attention for its unique segmented structure. Tunable soft and hard segment of PU makes it possible to tailor the mechanical properties, thermoplasticity and durability depending on the application of PU (Mi, *et al.*, 2014b). However, the lack of bioactivity limits the application of PU as a scaffold on its own. As a consequence, the introduction of Bioglass® into the PU is one of the alternatives to improve the bioactivity of the PU (Elbatal *et al.*, 2003; Ryszkowska *et al.*, 2010). Incorporation of Bioglass® is believed to be able to improve the mechanical properties as well as bioactivity of the polymer scaffolds. In order to fabricate PU reinforced Bioglass® scaffold, a fabrication process should be designed to optimize the porous structure of the scaffold. Therefore, solvent casting/particulate leaching (SC/PL) is one of the versatile techniques which is able to fabricate PU reinforced Bioglass® with adequate pore structure and porosity (Janik and Marzec, 2015).

2.2 Biomaterials and ideal scaffold requirement

Over the past years, thousands of surgeries were performed to replace or repair the damaged tissue due to trauma, diseases or injury. Most of the treatment concentrates on transplanting the tissue or organ from one part to different part of the body in the same patient (autograft) or from another person to the patient (allograft). While these progressive treatments are lifesaving, some major problems raised by these therapies. Costly, painful, anatomical restricted and high possibility to get infection disease from the donor-site are the problems associated with autografts treatments. Besides that, severe restrictions of allografts are the possibility of refutation by the patient's immune system and initiating infection or disease from the donor to the patient (O'Brien, 2011). Alternatively, the field of tissue engineering has emerged as a scientific field with the aim dedicated to the regeneration of functional human tissue. The term 'tissue engineering' was invented during National Science Foundation workshop in 1988 is defined as 'the application of principles and methods of engineering and life sciences toward the fundamental understanding of structure-function relationships in normal and pathological mammalian tissues and the development of biological substitutes to restore, maintain or improve tissue function' (O'Brien, 2011). Hence, the major goal in tissue engineering is to design biomaterial scaffolds that allow for cells to grow and consequently to generate the functional tissue in the host as an alternative to conventional organ transplantation and tissue reconstruction methods (Chen *et al.*, 2013).

In the scientific field of tissue engineering, biomaterial can be interpreted as a material used to replace or assist in the function of tissue while in intimate contact with it, either internally or externally (Chen *et al.*, 2013). Due to the rapid growth in orthopaedics treatments, research in biomaterials had now shifted from 'bio-inert'

towards ‘bioactive’ that integrate with biological molecules or cells and regenerate tissues (Stevens, 2008). According to the paradigm in tissue engineering, upon implant, the materials are resorbed and replaced over time while tissue regeneration occurred simultaneously. In general, bioceramics (Engin *et al.*, 1999), bioactive glasses (Hench, 2006), biological or synthetic polymers (Dhandayuthapani *et al*., 2011), and composite (Tetteh *et al.*, 2014) of these are the potential bioactive and biodegradable materials as a bone substitute.

The scaffold is a three-dimensional (3D) substrate for cells, and serves as a template for tissue engineering (Liu and Ma, 2004). In tissue engineering, fabrication of scaffold become an important study for tissue repair and regeneration. Hence, an optimal scaffold design for bone tissue engineering should fulfil several characteristics as follows:

1) Biocompatible

   The first requirement of any scaffolds is biocompatible, defined as the ability of a material to perform with an appropriate host response in a specific application (Anderson, 2006). Upon implanted, the scaffolds should not show any inflammatory or immunogenic reaction to the host tissue (Mahato *et al.*, 2017).

2) Bioactive and excellent biological properties

   Bioactivity resulted from surface modification of materials when exposed to interstitial fluids which able to form an interfacial bond with tissues leading to the formation of a firm bond of with hard and soft tissue (Livingston *et al.*, 2002). The properties of the implanted scaffold should

be suitable to promote cell attachment, proliferation and differentiation in order to permit new tissue formation (Asefnejad, *et al.*, 2011b).

3) Satisfactory porous structure

Pore size, porosity and interconnectivity are crucial structural properties for a porous scaffold that can influence the behaviour during in-vitro and in-vivo as well as mechanical properties. Macropore (>100μm) is fundamental for a good vascularization of the implant to allow blood vessels to reach the inner part of the scaffolds. Furthermore, the micropore (<100μm) creating roughness on scaffold walls, is an important feature to promote protein and cell adhesion in-vivo (Vitale-Brovarone *et al.*, 2009). High porosity (> 90%) is important for scaffolds for any tissue engineering applications including bone. Sufficient pore interconnectivity is required to ensure uniform cell seeding and distribution and the diffusion of nutrients to metabolites out from the cell/scaffolds construct (Jahan and Tabrizian, 2016). However, due to the diversity in bone features, cells and cytokines present, standard porosity and pore size cannot be set as a general guideline for optimal bone tissue scaffolds (Roohani-esfahani *et al.*, 2011).

4) Biodegradable

The main objective of tissue engineering is to regenerate the functional tissue in the host that eventually replace the degraded of an implanted scaffold over time. The scaffold and host bone should be bonded without the formation of scar tissue thus creating a stable interface. The degradation of the scaffolds should be resorbed at the same rate as bone regeneration. Besides, degradation product should be non-toxic and can be simply

excreted by the body so that the bone is able to reform in its original state and functional as living tissue (Jones *et al.*, 2006).

5) Adequate mechanical properties

Fabrication of porous scaffold with optimal mechanical strength is one of the major challenges in tissue engineering specifically for cardiovascular and orthopedic applications. Ideally, the scaffold should have matched mechanical properties with the tissue at the implantation site. The mechanical strength of the scaffold should be adequate to prevent cells from destructed by the compressive or tensile forces without restricting appropriate biomechanical prompts (Sokolsky-Papkov *et al.*, 2007). However, a developed scaffold with high strength restraints the scaffold to have high porosity which is important for vascularization and nutrients delivery. Hence, during the design of the scaffold, the mechanical properties and porosity should be balanced to ensure the scaffold is able to withstand the load during implantation while allowing for cell infiltration.

2.3 Biodegradable polymers for tissue engineering application

Polymeric materials have drawn great attention in the past few years due to their unique properties such as high surface-to-volume ratio, biodegradation, high porosity with very small pore size and tune able mechanical properties. In addition, the biodegradable polymer offered distinct advantages such as biocompatible, chemically versatile and adequate biological properties which are significant for tissue engineering application and organ substitution (Dhandayuthapani *et al.*, 2011). Biodegradable polymers are used for tissue engineering application should be biocompatible material, which does not cause potential immunological effect or

foreign body reaction. The degradation products of the chosen polymer should not be toxic and must be easily excreted by metabolic pathways (Liu and Ma, 2004). Both natural and synthetic polymers have been extensively investigated as biodegradable polymeric biomaterials. The biodegradation mechanism of polymeric biomaterials involves the cleavage of hydrolytically or enzymatically sensitive bonds in the polymer leading to polymer erosion (Katti *et al.*, 2002). Figure 2.1 shows the classification of biodegradable polymer into the natural and synthetic polymers.

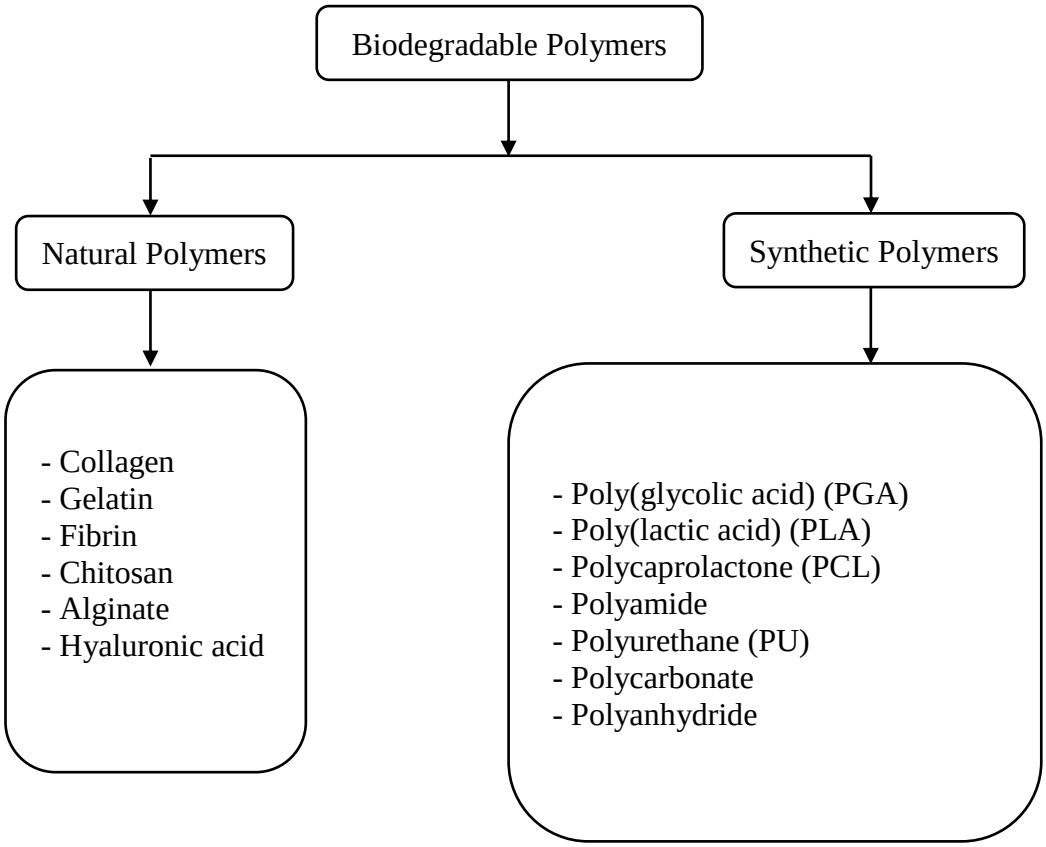


Figure 2.1 Classification of biodegradable polymers in tissue engineering

2.3.1 Natural polymers

Natural polymers can be considered as the first biodegradable biomaterials used clinically (Nair and Laurencin, 2007). Collagen which is one of the natural polymers that has been used biomedically for thousands of years (Ulery *et al.*, 2011). Naturally derived polymers possess distinct advantages which positively support cell adhesion and function (Liu and Ma, 2004; Armentano *et al.*, 2010), ability to mimic the microenvironment and ease of processing (Seal *et al.*, 2001). One of the extensive studies in natural polymer for tissue engineering is a collagen. Collagen is a fibrous protein majorly exist in skin and bone approximately for about 25% of the total dry weight of mammals. Molecular structure of collagen comprised of three α chains that assemble together. Each α chain is composed of more than thousands of amino acid (Parenteau-Bareil *et al*., 2010). Human-like blood vessel fabricated by a combination of collagen-chitosan tubular scaffolds showed superior biocompatibility with enhanced cell adhesion and proliferation. Besides, the scaffold achieved desirable levels of mechanical strength (Zhu *et al.*, 2009). Apart from collagen, gelatin played an important role as biomaterials. Gelatin is a natural material derived from collagen by hydrolysis and has an almost identical composition with the collagen (Harrington and Von Hippel, 1962; Liu *et al.*, 2009). The in-vivo study also showed gelatin sponge that had been isolated, cultured and seeded with rat bone marrow-derived a mesenchymal stem cells (MSCs) which was able to adhere, survive and proliferate. The transplanted gelatin sponge at the transected site of the rat spinal cord able to be biocompatible and incorporate completely with the spinal cord (Zeng *et al.*, 2011). Other biodegradable polymers such as chitosan (Zakhem *et al.*, 2012), alginate (Jeong *et al.*, 2012) and fibrin (Johnson *et al.*, 2010) salso howed positive biocompatibility during in-vivo studies. These studies proved, these natural polymers scaffold able to

promote cell adhesion during the in-vivo tests. However, fabricating the scaffolds from biodegradable natural polymers face a challenge with the poor mechanical properties which limits their use in load bearing application (O'Brien, 2011). Besides, the natural polymers exhibit immunogenicity and contains pathogenic impurities and less control over their biodegradability (Liu and Ma, 2004). As a consequence, a lot of studies focus on synthetic polymers for scaffolds application due to numbers of advantages including the ability to tailor mechanical properties and degradation kinetics to suit various application.

2.3.2 Synthetic polymers

Tailorable mechanical and physical properties of synthetic polymers such as tensile strength, elastic modulus and degradation rate of synthetic polymers can be reproduced under controlled conditions (Rezwan *et al.*, 2006). Poly(α-hydroxy esters) polymers, such as poly(glycolic acid) (PGA), poly(lactic acid) (PLA) and their copolymers, poly($_{DL}$-lactic-co-glycolic acid) (PLGA) are most frequently used in tissue engineering. These polymers have been revealed to be biocompatible and produce non-toxic degradation products with controllable degradation rate in-vivo. Hence, a long history as degradable surgical sutures resulted these polymers by having gained FDA (*US Food and Drug Administration*) approval for clinical use. These polymers degrade through hydrolysis of the ester bonds, with degradation products eventually removed from the body in the form of carbon dioxide and water. By altering chemical composition, crystallinity, molecular-weight value and distribution, the degradation rates of the synthetic polymers can be tailored depending on the requirements (Armentano *et al.*, 2010).

PGA is a rigid thermoplastic material with high crystallinity (46-50%), glass transition (36°C) and melting (225°C) temperature.Due to high crystallinity, PGA is not soluble in most organic solvents. PGA can be fabricated using the technique such as extrusion, injection and compression molding. However these techniques required careful control of processing condition due to high sensitivity of PGA to hydrolytic degradation (Gunatillake and Adhikari, 2003). Rapid degradation of PGA and insolubility in many common solvents caused a restriction to expend the study related with PGA-based which is only limited for sutures, drug delivery, biomedical devices and other short-term tissue engineering scaffolds. Rapid degradation causes loss of mechanical strength and significant formation of glycolic acid. Even though glycolic acid is bioresorbable by cells via the citric acid cycle, high level of glycolic acid has been related to a strong, undesired inflammatory response (Ulery *et al.*, 2011).

PLA is another well-known poly(α-hydroxy esters) polymers in tissue engineering application for its high biocompatibility and biodegradability properties as well as extensive mechanical properties profile. PLA is gained from lactic acid and converted back to the latter one when hydrolytically degraded. Lactic acid is one type of organic acid that can be produced by natural process like fermentation of sugars from natural resources such as sugarcane. Therefore, PLA can be synthesized and applied in an environmentally friendly cycle (Lasprilla *et al.*, 2012). The polymer is relatively hard, with the glass transition temperature within the range 60-70°C and melting temperature at 170-180°C. In addition, the key ability of PLA is ability to tailor its physical properties by material modifications (Gupta *et al.*, 2007). The degradation of PLA mainly occurs by hydrolysis after exposure to moisture for several months. There are two stages of PLA degradation. In the first stage, reduction in molecular weight due to random non-enzymatic chain scission of the ester group.

Then, followed by decomposition of the lactic acid and low molecular weight of oligomers by microorganism to produce carbon dioxide and water in second stage (Oyama *et al.*, 2009). The biocompatibility of the PLA has been long studied by the researcher. Majola *et al.* (1991) proved that the self-reinforced PLA implant in rats was biocompatible, slowly absorbable, and the implants possess sufficient mechanical properties for fixation of osteotomies. Medial femoral condyle osteotomies of sheep were fixed with PLA composites pins showed complete healing at the implant site without displacement or clinically relevant complications (Prokop *et al.*, 2005). In past recent years, many attempts have been done to improve the biocompatibility and bioactivity of the PLA by filling the polymer with bioactive ceramics or glass such as Bioglass® (Vergnol *et al.*, 2015), hydroxyapatite (HA) (Abdal-hay *et al.*, 2013) or tricalcium phosphate (β-TCP) (Lou *et al.*, 2014). Despite of excellent biocompatibility, processability and less energy dependence, the polymer also has certain drawbacks such as poor toughness with very brittle properties, slow degradation rate and relatively hydrophobic surface caused low cell affinity (Rasal *et al.*, 2010).

Although PGA, PLA and the copolymers had proved their ability as potential substitutes in tissue engineering, biodegradable PU has been extensively investigated as long terms medical implants. PU represent a diverse group of polymers that are linked by urethane bonds formed between isocyanates and hydroxyl group (Wolf *et al.*, 2015). PU has attracted great attention as it has unique segmented structure (Janik and Marzec, 2015). Tune able soft and hard segment properties (Mi, *et al.*, 2014b) of PU make it possible to tailor its mechanical properties, thermoplasticity and durability depending on its application (Bil *et al.*, 2009). Due to unique segmented properties of PU, the polymer is compatible to be applied for tissue engineering. Hence, this study

has chosen PU as the polymer matrix to be fabricated into a scaffold. Details description of PU will be elaborated in the next topic in this chapter.

2.3.3 Biodegradable polyurethane

For a few decades, polyurethane (PU) become a popular choice in biomedical applications, due to their outstanding biocompatibility, mechanical properties and versatility. Previous study had proved that biodegradable PU able to sustain cells ingrowth and controllable degradation into non-cytotoxic decomposition. These traits became a bonus for PU as new excellent candidates for tissue engineering scaffold due to tune able biological, physicochemical and mechanical properties. (Patel *et al*., 2011). Figure 2.2 represent the graphical abstract of PU as synthetic polymer in tissue engineering application.

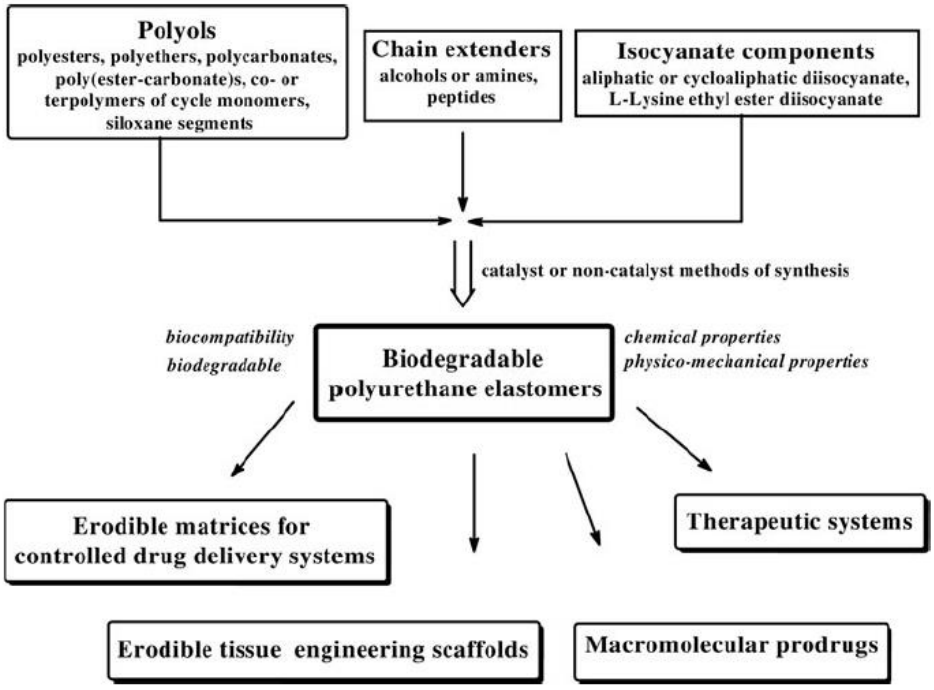


Figure 2.2 Graphical abstract of polyurethane in tissue engineering application (Sobczak, 2015)

2.3.3.1 Synthesize and chemistry of segmented structure of polyurethane

PU consists of urethane (-NH-CO-O-) linkage that was generated through the reaction of isocyanate with a hydroxyl group (Patel *et al.*, 2011). Generally, PU is synthesized with three main components: a difunctional polyol (macrodiol), a diisocyanate and a chain extender (usually a diamine or diol) by two step-polymerization. Controllable types of diisocyanate, polyol and chain extender determine the structure and properties of the PU. Low glass-transition temperature or high elasticity of synthesized PU is influenced by the soft segment. Meanwhile, the hard segments resulted in high glass-transition temperature, melting point or high strength of PU (Sobczak, 2015). The chemical structure of biodegradable PU is composed by block copolymers of (AB)n type which is consist of alternating polydisperse sections of hard segments (diisocyanate and low molecular diol chain extender) and soft segments (polyols) (Cauich-Rodriguez *et al.*, 2013). Figure 2.3 shows the standard two-step reaction to prepare segmented PU through polyaddition polymerization. In the first step, the soft segment polyol reacts with the hard segment diisocyanate along with the nitrogen gas flow to form a prepolymer. During this stage, the characteristic urethane linkage of PU are developed through the reaction between the hydroxyl-terminated end groups of the polyol and isocyanate. In the second step, the prepolymer segment is linked with low molecular weight chain extender to yield a high molecular weight of PU. Additional urethane functional groups are formed by using diol chain extender during this stage (Cauich-Rodriguez *et al.*, 2013).

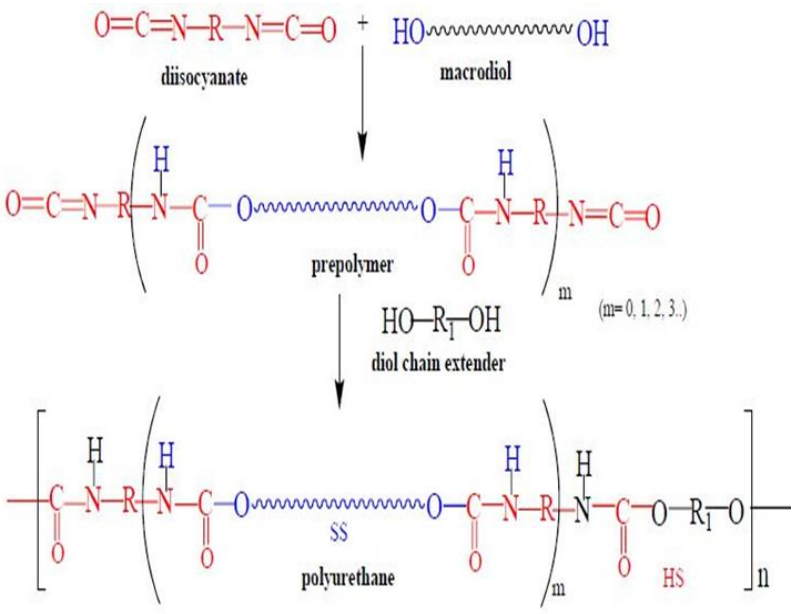


Figure 2.3 Standard two-step reaction to prepare segmented PU (Gunatillake & Adhikari., 2011)

Formation of hydrogen bonding is triggered between the isocyanate-derived groups which is urethane. Microphase separation in PU is generated by the hydrogen bonding and opposite polarity between the segments forming hard pseudo-crystalline domains and soft domains (Aldenhoff *et al.*, 2001). Thus, the structure of PU is composed by alternating soft amorphous segments constructed by long chain polyols and hard segment made up from diisocyanate and chain extender (Figure 2.4). Glass transition temperature belong to soft domains is below room temperature resulted with amorphous and elastomeric at room temperature. Therefore, hard domains acting as physical crosslinks that fix every soft segment at its two-end to avoid the chains from flowing apart under applied stress. The absence of chain flow makes the segmented PU to reshape elastically after released the stress (Guelcher, 2008). Besides, semi-crystalline of hard phase can be melted similar with other thermoplastic. In brief, segmented PU exhibiting thermoplastic characteristic with rubber-like mechanical

properties as well as able to melt at high temperatures and being soluble in polar solvents (Chen *et al.*, 2013).

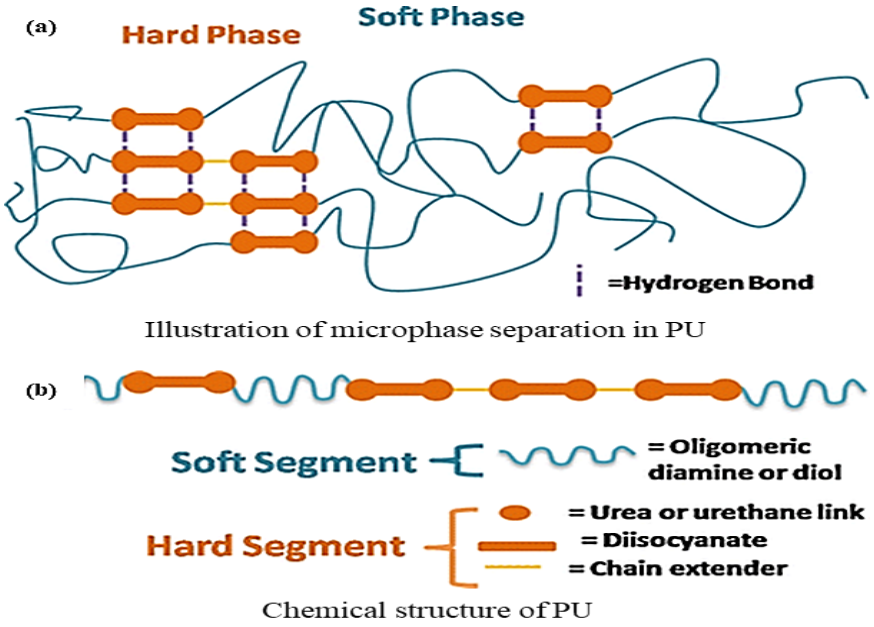


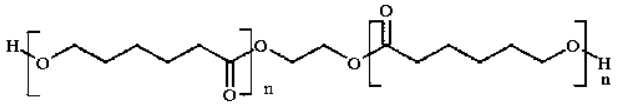
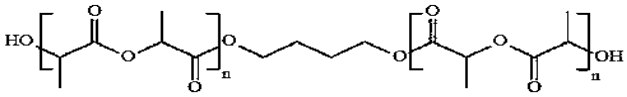
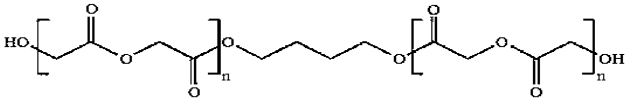
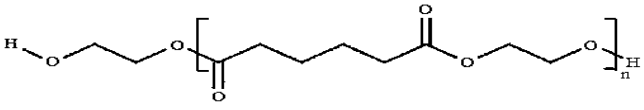
Figure 2.4 Illustration of (a) Microphase separation in PU and (b) Chemical structure of PU (He et al., 2014)

2.3.3.2 Soft segment of polyurethane

The soft segments part or known as polyol used in biodegradable PU are hydroxyl end functional groups (two or higher functionality). This segment had received great attention as it is the most vulnerable segment in the polymer. In synthesize of biodegradable PU, the typical polyols used as soft segment are polyesters, polyethers, polycarbonates and combination of these polyols in the form of diblocks and triblocks (Cooper and Guan, 2016). Generally, polyols possess low glass transition temperature which include in rubbery regime at the physiological temperatures. Hence, the type and length of polyol used influenced in physical properties of the biodegradable PU. For example, long polyols produced low-modulus PU elastomers and/or multifunctional polyols which resulted in rigid PU (Gisselfält and Helgee, 2003).

Polyesters (-R-CO-O-R'-) polyols based on caprolactone, glycolide and lactides to synthesize biodegradable PU have been investigated for tissue engineering purpose (Helminen *et al.*, 2003). This is mainly because of ester group is susceptible to chemical and enzymatic hydrolysis, a condition that is easily achieved in vivo (Chan-Chan *et al.*, 2010). Besides, polyester polyols offer excellent mechanical properties compared to other commonly used polyols for biomedical applications. Table 2.1 shows the list of common polyester diol structures in biodegradable PU. Among many type of the polyester diol, poly(ε-caprolactone) (PCL) diol is frequently used as soft segment (Bogdanov *et al.*, 1999) due to its enzymatic degradation, biocompatibility and slow hydrolytical properties (Ryszkowska *et al.*, 2010). Other typical polyesters such as those based on lactic and glycolic acids resulted in stiff and non-elastic of PU which are not preferable for soft and flexible tissue application such as cardiovascular, urological or gastrointestinal tissue (Trinca *et al.*, 2015).

Table 2.1 List of common polyester diol structures used in biodegradable PU (Cooper and Guan, 2016)

Chemical name	**Structure**
Poly(ε-caprolactone) (PCL) diol	
Poly(D,L-lactide) (PDLLA) diol	
Poly(glycolide) (PGA) diol	
Poly(ethylene adipate) diol	

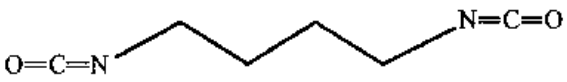
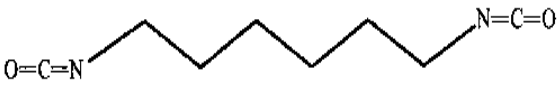
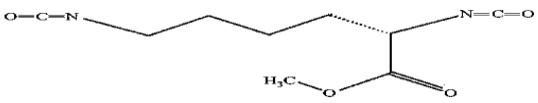
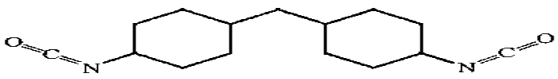
Momtaz *et al.* (2014) investigated the effect of changing the molecular weight of diol (2000, 3000 and 4000) on the properties of PU based on diphenylmethane diisocyanate, PCL and 1,4-butanediol. The results showed no crystalline structure of the soft segment was detected in the 2000 series, whereas 3000 and 4000 series present crystalline soft domain. Meanwhile the shape memory effect on the polymer revealed that increased in the soft segment length showed better shape fixity while high hard segment content resulted in better shape recovery. Most of the biodegradable synthetic polymer including PU based on polyester diol consist of rigid polyester chain and high crystallinity. These properties are lack in elasticity for most of the scaffolds for human tissue application. Hence, Mi *et al.* (2018) synthesized PU based on PCL triols combined with polycaprolactone-block-polytetrahydrofuran-block-polycaprolactone (PCTC) tri-block copolymer in order to introduce long chain crosslinking which provide more flexibility to the molecular chain. As a result, the synthesized PU achieved 99.8% maximum recovery rate. Moreover, fabricated scaffold from this PU stimulate the proliferation of different type of cells during in-vitro cell culture.

2.3.3.3 Hard segment of polyurethane

In formulating biodegradable PU, the most widely studied isocyanate are aromatic, aliphatic and cycloaliphatic diisocyanate. Aromatic isocyanate such as 4,4'-diphenylmethane diisocyanate (MDI) and toluene diisocyanate (TDI) yield the highest tensile properties with high melting temperatures, low cost and high reactivity (Cooper and Guan, 2016). However, these diisocyanate are not suitable for biomedical application due to potential toxicity issues related with aromatic diamines formed as one of the degradation products (Tosin *et al.*, 1998). Therefore, aliphatic diisocyanate including 1,4-butane diisocyanate (1,4-BDI), 1,6-hexamethylene diisocyanate (1,6-

HDI) and L-Lysine methyl ester diisocyanate (LDI) as well as cycloaliphatic diisocyanate such as 4,4'-dicyclohexamethylene diisocyanate (HMDI) and isophorone diisocyanate (IPDI) have become the choice of isocyanate to synthesize biodegradable PU that fit for biomedical application (Sobczak, 2015). Table 2.2 illustrates the chemical structure of both aliphatic and cycloaliphatic diisocyanate.

Table 2.2 Chemical structure of aliphatic and cycloaliphatic isocyanate (Cooper and Guan, 2016)

Chemical name	Structure
1,4-butane diisocyanate (1,4-BDI)	
1,6-hexamethylene diisocyanate (1,6-HDI)	
L-Lysine methyl ester diisocyanate (LDI)	
4,4'-dicyclohexamethylene diisocyanate (HMDI)	

1,4-BDI and 1,6-HDI are two most common use diisocyanate in biodegradable synthesize to circumvent any potential toxicity concerns. Some study had proved that 1,4-BDI is considered biocompatible as the degradation products as the hydrolyzed product yield putrescine which is naturally occurring in the body (Zuidema *et al.*, 2009). A family of poly(ester-urethane) urea had been synthesized from PCL, 1,4-BDI and Lysine ethyl ester (Lys) or putrescine as chain extender. Both type of PU resulted in high flexibility with breaking strain 660-895% and tensile strength from 9.2-29 MPa. Meanwhile, the polymer showed degradable ability with 10-50% of mass loss in