SCHOOL OF MATERIALS AND MINERAL RESOURCES ENGINEERING

UNIVERSITI SAINS MALAYSIA

FABRICATION AND CHARACTERIZATION OF POLYLACTIC ACID (PLA) BLEND BIOMATERIAL USING 3D PRINTING FOR POTENTIAL APPLICATION IN TISSUE ENGINEERING

by

CHOW LI CHIN

Supervisor: Dr. Zuratul Ain Abdul Hamid

Dissertation submitted in fulfillment

of the requirements for the degree of Bachelor of Engineering with Honours

(Polymer Engineering)

Universiti Sains Malaysia

JUNE 2018

DECLARATION

I hereby declare that I have conducted, completed the research work and written the dissertation entitled: "Fabrication and Characterization of Polylactic Acid (PLA) Blend Biomaterial using 3D Printing for Potential Application in Tissue Engineering". I also declare that it has not been previously submitted for the award of any degree or diploma or other similar title of this for any other examining body or university.

Name of Student : Chow Li Chin Signature :

Date :

Witness by,

Supervisor : Dr. Zuratul Ain Abdul Hamid Signature :

Date :

ACKNOWLEDGEMENTS

First of all, I wish to thank God for giving me the opportunity to embark on my Final Year Project (FYP) and for completing this long and challenging journey successfully.

Next, my appreciation goes to the USM Engineering Campus School of Materials and Mineral Resources Engineering for giving me chance to gain valuable practical experience and knowledge in completing this final year project.

Successfully completion of this project requires helps from a number of persons. I would like to express my special thanks and appreciation to project supervisors, DR. Zuratul Ain Abdul Hamid for giving me such attention and supervision during period of my research work. Her patience and generosity in guiding and inspiring me throughout this whole project period are really meaningful.

Moreover, a big contribution and hard worked from all technical staff in laboratory. This project would have never been completed without their guide and assistance. Special thanks also to my colleagues and friends for helping thorough the journey.

Last but not least, I would like to thank my family members for their endless support, physically and mentally. Not forgetting every individual who is involved, your help and kindness will be remembered.

Thank you.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

3D: Three dimensional

AM: Additive manufacturing

ASTM: American Society for Testing

ATBC: Acetyl tri-n-butyl citrate

CAD: Computer-aided design

DSC: Differential scanning calorimetry

ECM: Extracellular matrix

FDA: Food and Drug Administration

FDM: Fused deposition modeling

FTIR: Fourier transform infrared spectroscopy

HA: Hydroxyapatide

MSDS: Material Safety Data Sheet

OLA: Oligomeric lactic acid

PEG: Polyethylene glycol

PEO: Polyethylene oxide

PES: Polyethylene glycol-succinate

PLA: Polylactic acid

PVA: Polyvinyl acetate

SEM: Scanning electron microscopy

SLS: Selective laser sintering

TE: Tissue engineering

TEG: Trriethylene glycol

LIST OF SYMBOLS

ΔH_{cc}: Enthalpy of crystallization at crystallization temperature

ΔH_m: Enthalpy of fusion at melting temperature

ΔH^o_m: Heat of fusion of a perfect orthorhombic PLA crystal

g/cm^3: Grams per cubic meter

J/m^2: Joule per meter square

MPa: Mega pascal

rpm: Revolutions per minute

T_{cc}: Cold crytsallization temperature

T_g: Glass transition temperature

T_m: Melting temperature

wt%: Weight percentage

PENYEDIAAN DAN PENCIRIAN POLILAKTIK ASID (PLA) DENGAN TEKNIK PERCETAKAN TIGA DIMENSI (3D) UNTUK APPLIKASI DALAM KEJURUTERAAN TISU

ABSTRAK

Kajian ini bertujuan untuk mengkaji kesan pemplastik kimia kepada sifat-sifat mekanik polilaktik asid (PLA) yang dihasilkan daripada teknik percetakan tiga dimensi (3D), supaya berpotensi digunakan sebagai bahan perancah dalam kejuruteraan tisu. Dalam kajian ini, PLA bercampur dengan pemplastik kimia seperti polietilena glikol (PEG) atau trietilena glikol (TEG) dengan pelbagai kepekatan (0, 5, 10, 15, 20, 25 wt%) menggunakan mesin ekstruder skru tunggal. Dengan penambahan pemplastik tersebut ke dalam PLA, sifat-sifat keliatan polilaktik asid diperbaiki. Namun, daya tegangan dan lenturan berkurangan kalau berbanding dengan PLA yang tulen. Polilaktik asid mencapai sifat-sifat mekanik yang optimum pada kepekatan 10 wt% dan 15 wt% bagi PEG dan TEG. Fourier transform inframerah (FTIR) spektroskopi mendedahkan interaksi fizikal antara PLA dengan pemplastik tersebut. Kalorimetri pengimbasan perbezaan (DSC) mendedahkan penurunan dalam suhu peralihan kaca apabila kepekatan pemplastik bertambah. Namun demikian, fenomena permisahan fasa telah ditemui apabila PEG dan TEG ditambah melebihi 10 wt% dan 15 wt%. Mikroskopi imbasan elektron (SEM) mendedahkan ketulan pemplastik dalam PLA pada kepekatan yang tinggi. Selain itu, sifat-sifat mekanik yang diperoleh daripada spesimen bercetak pencetak tiga dimensi adalah lebih rendah berbanding dengan teknik acuan mampatan. Walau bagaimanapun, PLA yang dihasilkan dalam kajian ini dengan penambahan pemplastik telah mencapai sifat kebolehlenturan yang diingini untuk menyokong pertumbuhan semula tisu dalam perancah yang terbentuk.

FABRICATION AND CHARACTERIZATION OF POLYLACTIC ACID (PLA) BLEND BIOMATERIAL USING 3D PRINTING FOR POTENTIAL APPLICATION IN TISSUE ENGINEERING

ABSTRACT

In this study, it was to study the effect of plasticizer on the mechanical properties of three-dimensional (3D) printed polylactic acid (PLA) blend system, for potential application as scaffolding material in tissue engineering. The PLA was plasticized with Polyethylene glycol (Mw-6,000 g/mol) or Triethylene glycol (Mw-150 g/mol) in various plasticizer concentrations (0, 5, 10, 15, 20, and 25 wt%) using single screw extrusion. With the incorporation of such plasticizer into PLA matrix, the toughness properties e.g. elongation at break and impact strength of PLA specimen improved due to reduce in stiffness of plasticized PLA. Nevertheless, the tensile and flexural strength decreases, as compared to neat PLA. In related to plasticizer content, the plasticized PLA achieved optimum mechanical performance at 10 wt% and 15 wt% respectively for PEG and TEG. Fourier transform infrared (FTIR) spectroscopy reveals the physical interaction between PLA and its plasticizer. Differential scanning calorimetry (DSC) study reveals decrease in glass transition temperature as concentration of plasticizer increases. However, some extent of phase separation has been found when PEG and TEG incorporated greater than 10 wt% and 15 wt% respectively. Scanning electron microscopy (SEM) observations reveals the plasticizer lump in the PLA matrix at high concentration of plasticizer. Besides, the mechanical properties obtained from 3D printed specimen were lower than compression molding technique. However, the developed 3D printed plasticized PLA had achieved desired mechanical flexibility improvement with acquired strength for support tissue regeneration in scaffold.

CHAPTER 1 INTRODUCTION

1.1 Background

Tissue is defined as a bunch of cells that have similar morphological features and perform a specific function in a body. For instance, articular cartilage tissue is a functional connective tissue that surrounding the surfaces of articulating joints in the body. The occurence of unexpected incidents e.g. injuries, disease, or trauma possibly in causing a degeneration or damage of tissues cells in the human body. Eventually, it may lead to organ failure or malfunction since these functioning tissue cells were loss (Birla, 2016).

The common clinical therapies for damaged tissues were nonsurgical interventions (e.g. physical treatment, pain-control) and surgical techniques (e.g. autografts, allografts implantation). Although these techniques would improve the life quality of patients, but it was not capable of regenerate of functional tissues akin to the origin tissue and are often highly case dependent. Moreover, the shortage of tissue or organs for implantation due to lack of organ donor, and the problems associated with the organ transplantation e.g. donor site morbidity or pathogen transfer, eventually will lead to the emergence of tissue engineering in this recent year (Shi, 2006).

Tissue engineering (TE) is introduced as an alternative way besides tissue or organ implantation. The ultimate-goal of TE is to regenerate the anatomic structures and recover the functions of the damage tissue or organs through the fabrication of biocompatible three-dimensional (3D) artificial tissue or organs like the heart, liver, and kidney as substitute (Birla, 2016).

Most of the tissues in body is complex and exists in 3D porous structure. For instance, the cartilage tissue is avascular, and not innervated. Despite the lacking blood vessels and nerves in cartilage tissues, and it comprised of only one cell type, hence it does not need vascularization or multiple cell-cell interactions. This making tissues engineering became an attractive potential method for cartilage regeneration due to easier to create the microenvironment for cell growth and proliferation (L.G Zhang, 2016).

Most of cells in body are residing in a solid matrix known as extracellular matrix (ECM). Hence, the tissue engineering field was study on the use of porous 3D scaffolds to create an appropriate ECM microenvironment for the regeneration of tissues and organs. An ideal scaffolds should acquire some characteristic e.g. non-toxic, biodegradable, desirable mechanical properties for acting as template, high porosity for cell growth and migration, and effective transports of substance like nutrients, oxygen, and waste, as well as the growth factors (O'Brien, 2011).

The researchers proposed the use of rapid prototyping technology (also known as 3D printing) to fabricate a customized tissue scaffold with controlled size (micro-scale) and good pore morphology. It is a computer-aided fabrication technique. There are more than 40 different types of 3D-printing techniques available in market. Among these technique, the Fused deposition modeling (FDM) was the most popular for scaffolds manufacturing because it able to process plastics materials through layer-adhesion of the filaments. Figure 1.1 illustrated the manufacturing process for a porous tissue scaffolds using a 3D printing technique (An, Teoh, Suntornnond, & Chua, 2015).

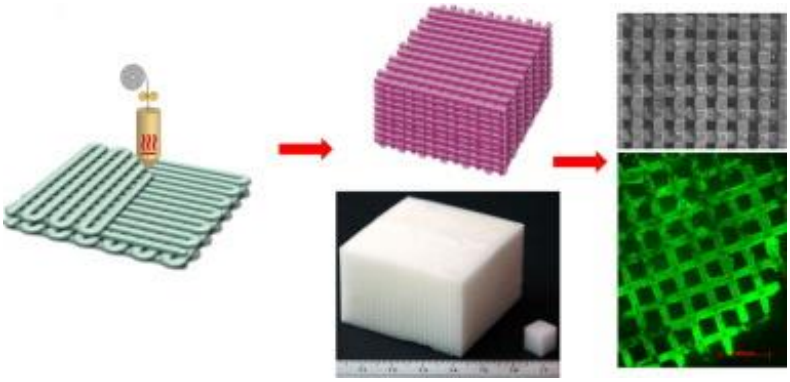


Figure 1.1 Three-Dimensional Printing of Tissue Scaffolds (Mohanty et al., 2015)

Among the types of biodegradable polymer available in market, polylactic acid (PLA) appears to be the most common biomaterials used for scaffold manufacturing. Polylactic acid is belong to the family of aliphatic polyesters, and it is made up by the lactic acid (2-hydroxy propionic acid, LA) building block. The basic building block of PLA can be produced either from fermentation of renewable natural sources (mainly starch and sugar feedstock, corn and rice), or through chemical synthesis. Due to production from these natural sources, PLA is considered as a bio-active substance to human body (Garlotta, 2002). Figure 1.2 shows the skeletal structure of polylactic acid.

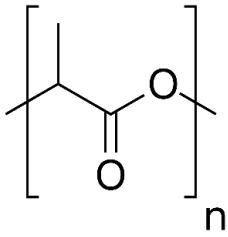


Figure 1.2 Skeletal Structure of Polylactic acid (Gupta and Kumar, 2007)

Besides, polylactic acid (PLA) classified as a biodegradable plastic due to presence of hydrolysable ester functional group in its structure. It only degrades into water and carbon dioxide (CO_2), both of these substances are neither toxic or carcinogenic to human body when implanted (Xiao et al., 2012).

1.2 Problem Statement

Although tissue generation procedures have achieved a large improvement in recent decades, however, it still remaining a major challenge to fabricate an ideal tissue engineering scaffolds from a 3D printing technology.

Polylactic acid (PLA) is a recognized biomaterial used for scaffolds production using a 3D printing technology due to its unique biocompatible and biodegradable properties. In comparison, it has a relatively higher tensile strength than others biodegradable materials. However, the major limitation of polylactic acid is its inherent low impact properties. Polylactic acid is very stiff material with less than 10% elongation at break. It becomes rigid and brittle near the room temperature due to its glass transition temperature (T_g) of about 55^{o}C. This will cause PLA has a poor impact strength, and tend to produce a catastrophic failure (cracking). This had limits its application to use as scaffolds material which require to regenerate the extracellular matrix (ECM) environment in human body which is flexible in nature (Rasal et al., 2008).

In previous research work, the limitation of polylactic acid (PLA) was improved by blend PLA with other types of soft-polymer or plasticizer. For instance, the impact strength or elongation at break was improved through incorporation of plasticizer into PLA (Xiao et al., 2012) like poly(vinyl acetate)(PVA) (Pennings et al., 1996), citrate ester (Labreque et al., 1997), oligomeric lactic acid (OLA) (Martin and Averous, 2001), acetyl tri-n-butyl citrate (ATBC) (Scandola et al., 2003), polyethylene glycol (PEG) (Hassouna, Raquez et al. 2011). Beside from plasticization, PLA also blend with others soft polymer e.g. block copolymer of ethylene glycol and propylene glycol (Pluta and

Piorkowska 2015), polyethylene glycol-succinate copolymer (PES) (Avolio, Castaldo et al. 2015).

Unfortunately, majority of these studies were focus on improving polylactic acid (PLA) mechanical properties for used in blow-filming or injection molding application such as film, automotive parts, etc, instead of biomedical application. Even there are some research studied on PLA blend used in biomedical application such as tissue scaffold, but mostly was through others fabrication technique such as solvent casting, phase separation, etc. Currently, the PLA blend developed was not used for 3D printed scaffold in tissue engineering. Besides, the compatibility between plasticizer and polymer matrix also would further limit the research.

Hence, it is considered a potential research and challenge in developing a polylactic acid (PLA) blend material for 3D printing biomedical application using a polyethylene glycol (PEG) or triethylene glycol (TEG) as plasticizer through 3D printing technique. The blended material was aimed to exhibit an optimum toughness and strength properties so that it able to extrude into filament without breakage; meanwhile, it must give a positive performance during application e.g. in tissue scaffold that have a high demand in toughness properties.

1.3 Research Aim and Objectives

The main aim of this project was to develop a polylactic acid (PLA) blend material that exhibit optimum strength and toughness properties, so that it suited for used for biomedical application e.g. tissues scaffold. In this research, several objectives are set to achieve the goal:

i. To study the effect of different types of plasticizers (PEG and TEG) on the mechanical properties of Fused deposition 3D printed PLA blends materials.

ii. To investigate the effect of different loading of plasticizers (0, 5, 10, 15, 20, and 25 wt%) on the mechanical properties of 3D printed PLA blends materials.

iii. To compare the effect of different fabrication technique (3D printing and compression molding) on the mechanical properties of PLA blends materials.

1.4 Significant of Work

In this study, the mechanical properties of Polylactic acid (PLA) were modified with incorporation of plasticizer. The types of plasticizer studied were polyethylene glycol (PEG) and triethylene glycol (TEG), which molecular weight are 6000 g/mol and 150 g/mol respectively. The uses of these plasticizers were due to its biocompatibility properties approved by us Food and Drug Administration (FDA). The focus of this study is to determine the effect of those plasticizer in modifying the properties of PLA. Polyethylene glycol (PEG) is considered a common plasticizer used in PLA blend in previous study. However, PEG exhibit some limitation in enhancing the mechanical performance. Hence in this study, another cheaper and low molecular weight plasticizer called triethylene glycol (TEG) is used for modifying the PLA properties also. Besides, the influence of loading percentage of these plasticizer also investigated.

All the mechanical properties of plasticized polylactic acid (PLA) are characterized through several standard mechanical testing methods consist of tensile (ASTM D-638), flexural (ASTM D-790), and impact (ASTM D-256) testing. Others tests such as Fourier Transform Infrared (FTIR), Differential Scanning Calorimetry (DSC) and Scanning Electron Microscopy (SEM) used for assessing the blend properties of PLA such as chemical, thermal, and morphological respectively. Based on the characterization test, the effect of such plasticizer incorporated in PLA blend on modifying the mechanical strength and toughness will be compared. Besides, the optimum loading percentage for each plasticizer types that results in better mechanical performance for potential used in producing of tissue scaffold will be investigated.

CHAPTER 2 LITERATURE REVIEW

2.1 Development of Tissue Engineering (TE)

In the biological term, tissue is made up by a group of specialized cells that share similar morphological features and to perform a specific function in a body. In general, the four most common tissues in human body are muscle, epithelial, connective and nervous tissues. The nervous tissue is responsible for receiving and transmitting impulse, and connective tissue is for support body movement, while epithelial and muscle tissue giving protection to body and create motion respectively. The combination of multiple types of tissues would become organs, which like brain, bones, skin, etc. (Birla, 2016). Figure 2.1 illustrates the types of tissue in human body.

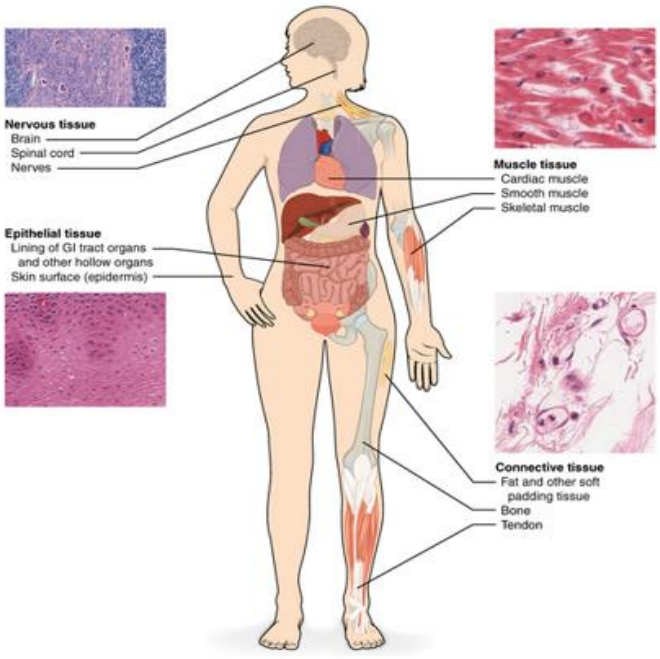


Figure 2.1 Types of Tissue in Human Body (Birla, 2016)

The happens of unexpected incident e.g. injuries, disease, or trauma possibly in causing a degeneration or damage of tissues cells in the human body. Eventually, it may cause an organ failure or malfunction due to loss of these functioning tissue cells (Lanza, Langer, & Vacanti, 2013). In the past decades, surgical reconstruction or transplantation of organ defect are the only way. However, due to lacking of quality and amount of autologous grafts, also the problems associated with their transplantation such as donor site morbidity, immune rejection, and pathogen transfer, eventually lead to the introducing of the discipline of tissue engineering field (Shi, 2006).

The concept of tissue engineering (TE) was arises in year 1993 and introduced by Langer and Vacanti. Tissue engineering can consider as the second generation of biomimetic science, or advance stage of biomaterials science. The main objective of tissue engineering is to regenerate and even recover the anatomic structures and functions of damage or injured tissue and organs using modern technique (Birla, 2016).

2.1.1 Introduction of Tissue Scaffolds

There was various technique used for tissue regeneration; but each of them incorporated with limitation. A research had reported that the isolated chondrocytes in cartilage tissue have a low proliferation rate and possibly experience dedifferentiation rapidly after expansion. In order to maintain desired cell phenotypes and to guide the cells form the functional tissue structure, the concept of three-dimensional (3D) scaffolds introduced in the field of tissue engineering (L.G Zhang, 2016). Scaffold is defined as an artificial extracellular matrices (ECM) or template which support three-dimensional tissue formation. It can be engineered to cause a desirable cell interactions, as result contribute to the formation of new functional tissues in body (Birla, 2016).

(a) Working Principle of Tissue Scaffolds

In tissue engineering, the fundamental principle of regeneration of artificial new tissues or organs are using the scaffolds are through obtaining few pieces of living tissue from the injured part (e.g. bone, cartilage, skins) either from the patient or from universal cell source, then disassembling the tissue into cell suspension to expand them to a certain concentration. After, the cells are seeding into 3D-scaffolds, growth factors and dynamic forces are supplied to the cells. Next is engineered the artificial tissue and organs with the anatomically desired architecture to recover the lost body part functions. At last, implanted the artificial tissues or organ into the patient injured part (Shi, 2006). Figure 2.2 below demonstrated the process flow for tissue regeneration.

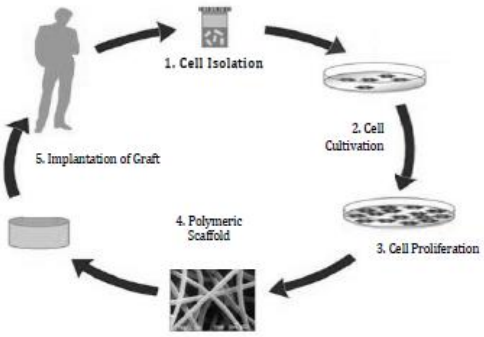


Figure 2.2 Process Flow for Tissue Regeneration in Tissue Engineering

In 2009, there was a new improvement achieved by the field of tissue engineering. A team led by the thoracic surgeon had success in implanted the world's first bioartificial transplant into a patient which awaiting tracheal reconstruction. In this surgical, a porcine jejunal segment living tissue was obtained from a pig which received human care in compliance with the health regulation. Next, the obtained living tissue was reseeded into a bioartificial vascularized scaffold with a dynamic tissue culture. The engineered tissue was then characterized according to its histology, immune-

histology, metabolic activity and life-dead assay before implant into human body (Mertsching et al., 2009).

(b) Characteristics of Tissue Scaffolds

In tissue engineering, an ideal 3D printed scaffolds should able to meet both biophysical, biomechanical, and biochemical requirement. In term of biophysical, scaffolds are currently being developed in a three-dimensional (3D) way to provide better mimicking the ECM environment for cell regeneration purpose since the cells in the body grow within an organized 3D extra-cellular matrix (ECM), surrounded by other cells. The scaffolds must provide structural and mechanical support, also supply sufficient nutrient, so that it enables growth and migration of cells to form a functional tissue that can actively remodel once implanted (Cristina C, et al, 2013).

In term of biomechanical, the cells are able to sense the matrix stiffness, which results in mechanical signalling. Cell will contract to pull on the matrix to which they are attached in routinely, and generating an internal tension. This mechanical stimulus is then converted into a chemical response which would influence directly on cell differentiation (Cristina C, et al, 2013).

The interactions between cell-cell and cell-ECM can affect the cell proliferation and differentiation process. Hence in term of biochemical, the 3D scaffolds should design in a highly porous structure, well-connected network, and also have consistent and adequate pore size (>300μm) to allow cell migration and infiltration. Besides, the binding of soluble Growth Factors (GF), enzymes and other effector molecules e.g. nutrient and oxygen, will controlling their diffusion and local concentrations (Cristina C, et al, 2013).

2.2 Fabrication Technologies of Tissue Scaffold

Since the development of tissue engineering, a number of conventional manufacturing techniques developed for fabrication of porous 3D scaffolds i.e. solvent casting, phase separation, fiber bonding, etc.

(a) Solvent casting/particulate leaching

For a solvent casting, or also called particulate leaching, it was common method used to fabricate scaffolds for tissue engineering applications. *Huang et.al* carried out this method by ground the salt NaCl powder and then screen with a 109 micrometer sieve to remove the smaller particles. The sieved salts are paved on a petri dish and allowed to form a layer of approximately 1.5 mm thickness. The salt-solution compound is then placed in a vacuum for 12 hours to remove the solvent. The dried polymer-salt composite is then placed in water for 48 hours for leaching out the porogens. The leached samples are freeze-dried for 4 hours and stored in vacuum prior to use. Figure 2.3 showed the processing for solvent casting (Ma, 2004).

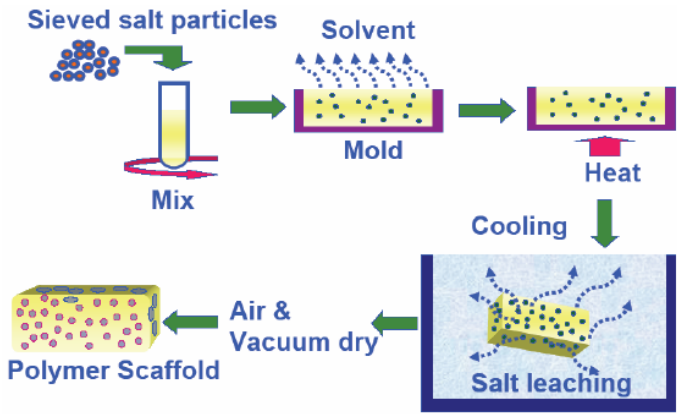


Figure 2.3 Solvent Casting Process for 3D Scaffold Fabrication

The solvent casting process is easy to carry out. It able to control the pore size. However, the pore shape and inter-pore openings are not controlled in this method. This technique can be further improved via a fused salt templating technique. As reported by *Gao.J*. in his research, it used sodium chloride (salt) crystals as the solid porogens, and were packed into a mold and then fused in a humid chamber. After, the polyglycerol sebacate (PGS) (biodegradable elastomer) was cured and dispersed throughout the whole fused salt template. The dissolution of the salt and subsequent lyophilization would produce elastomer sponges with approximately 90% porosity, interconnected macropores (75-150 micron), and extensively micropores (5-20 micron)(Gao, Crapo, & Wang, 2006). However, this technique exhibited a less organized pore structure and also have a large value of small interconnects. The interconnect diameter about doubled after a fusion time of 24 hours (Mattiasson, Kumar, & Galeaev, 2009).

(b) Phase separation

In phase separation method, the polymer solution is separates into two phases, a polymer-rich phase and polymer-lean phase. After the solvent is removed, the polymer-rich phase solidifies. Phase-separation techniques have been widely used to fabricate a porous membrane for filtration. However, the pores formed in this techniques usually not uniformly distributed and have a large diameter, hence not appropriate to be use for tissue engineering application (Ma, 2004). Figure 2.4 showed the processing for solid-liquid phase separation for scaffold fabrication.

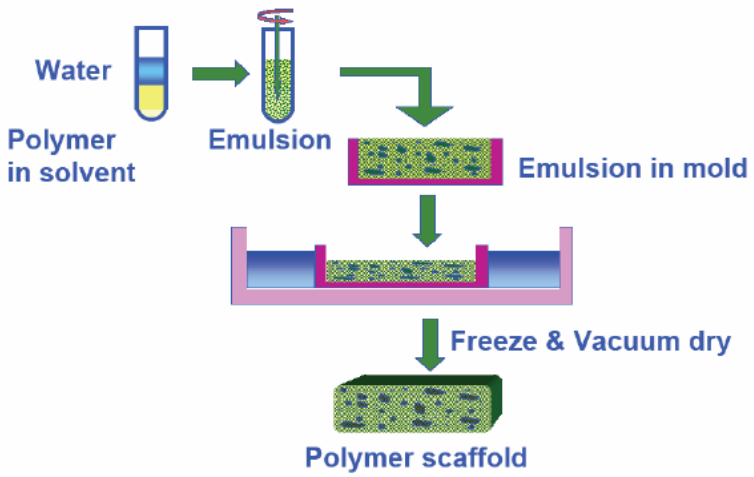


Figure 2.4 Solid-Liquid Phase Separation Process for 3D Scaffold Fabrication

(Liu & J. Webster, 2007)

(c) Textile technologies

By using textile technologies, polylactic acid and other semi-crystalline polymers can be processed into fiber. The PLA nonwoven scaffold have been used either alone or combined with other biodegradable polymer for engineering of tissue or organs. However, there are few disadvantages of PLA nonwoven scaffolds, such as poor mechanical strength, high degradation rate, limited fiber diameter variations, and difficulty in controlling the desired microarchitectural e.g. pore geometry (Mohanty et al., 2015).

In summary, although the mentioned processing techniques are quick and economical, but all these methods do not enable an accurate control of microarchitectural details such as geometry, pore size, and their interconnections and distribution within the scaffolds.

2.2.1 Introduction of Three-Dimensional (3D) Printing Technology

To overcome such problem, researchers proposed the use of computer-aided design (CAD) techniques to fabricate the scaffolds. Hence, the 3D printing technology was introduced. The 3D printing technique have existed since the 1980s. The first patent for the 3D printer was issued by *Charles Hull* in 1986. Hull made 3D printing history by inventing stereolithography which allowed designers create 3D tangible object using digital data. This technique has become a popular and advantageous way to create 3D scaffolds due to it high design flexibility using a CAD digital model. It has highly reproducible well-controlled architecture (micro-scale size, shape, porous structure, branching, geometry, and orientation) and materials compositional variations.

The 3D printing technique able to achieve topographically appropriate structures in the different zones, hence it can provide right cues to guide cell growth and alignment, induce cell proliferation, and facilitate the tissue regeneration. Besides, by combine with the imaging techniques, 3D printing able to produce customize scaffolds for particular application or for specified patient (L.G Zhang, 2016).

Conceptually, 3D printing technology is an approach where 3D designs can be built directly from a Computer-Aided Design (CAD) file without any part-specific tools. While other conventional manufacturing process require a careful and detailed analysis of the part geometry to determine types of tools and processes must be used, and additional fixtures may be required to complete the part. In contrast, 3D printing needs only some basic dimensional details and a small amount of understanding as to how the machine works and the property of materials that are used to build the part (Rodrigues, Benning, Ferreira, Dixon, & Dalgarno, 2016).

Table 2.1 summarizes some of additive manufacturing (AM) techniques used for tissues engineering application including its advantages and disadvantages:

Table 2.1 Additive Manufacturing Technique for Tissue Scaffolds Application

Technique	Process details	Processed materials for tissue engineering	Advantages (+) and disadvantages (−)
Fused Deposition Modeling (FDM)	• Filament of heated polymer extruded through nozzle	• Polylactic acid (PLA) • Acrylonitrile butadiene styrene (ABS) • Polypropylene (PP) • Polycaprolactone (PCL)	+: No need for platform −: Material restriction due to need for molten phase
3D Plotting/direct ink writing	• Strands of paste/viscous material extrusion based on the predesigned structure • Layer by layer deposition of strands at constant rate, under specific pressure • Disruption of strands according to the tear of speed	• Polycaprolactone (PCL) • Hydroxyapatite (HA) • Bioactive glasses • Mesoporous bioactive glass/alginate composite • Polylactic acid (PLA)/polyethylene glycol (PEG) • PLA/(PEG)/G5 glass • Poly(hydroxymethylglycolide-co-ε-caprolactone) (PHMGCL)	+: Mild condition of process allows drug and biomolecules (proteins and living cells) plotting −: Heating/post-processing needed for some materials restricts the biomolecule incorporation

…Continued

Laser-assisted bioprinting (LAB)	• Coating the desired material on transparent quartz disk (ribbon) • Deposition control by laser pulse energy	• HA • Zirconia • HA/MG63 osteoblast-like cell • Nano HA • Human osteoprogenitor cell • Human umblilical vein endothelial cell	+: Ambient condition Applicable for organic, inorganic materials and cells −: Homogeneous ribbons needed
Selective Laser Sintering (SLS)	• Preparing powder bed • Layer by layer addition of powder • Sintering each layer according to the CAD file, using laser source	• PCL • Hydroxyvalerate) (PHBV) • Poly(L-lactic acid) (PLLA)	+: No need supports No post processing −: Feature resolution depends on laser beam diameter
Stereolithography (SLA)	• Immersion of platform in photopolymer liquid • Exposure to focused light according to desired design • Polymer solidifying at focal point,	• Poly(propylene fumarate) (PPF)/diethyl fumarate (DEF) • PPF/DEF-HA • PDLLA/HA	+: Complex internal features can be obtained Growth factors, proteins and cell patterning is possible −: Only applicable for photopolymer

Out of those techniques stated in Table 2.1, Fused Deposition Modeling (FDM) 3-D printing technology appeared to be the most popular method for tissue scaffolds manufacturing. A fused deposition modeling (FDM) 3D printing offers the ability to directly print 3D porous scaffolds with pre-designed shape, solvent-free, controlled porous size and interconnected porosity. It is works with thermoplastics material such as ABS, PLA and HDPE etc. In this method, the materials changes from solid state e.g. filament form to semi-liquid state during the extrusion process to form layers upon layers. Each new layer will then stack on top and fused with the previous layer immediately when the material is almost hardened. It was more appropriate used for scaffolds fabrication since it involves the stacking of porous bar in scaffold to create a porous three-dimensional structure in final (Haruna, 2014).

(a) Fabrication of Tissue Scaffolds via Fused Deposition Modeling (FDM)

The printing process in Fused Deposition Modeling (FDM) 3D printing technique machine can be divided into 3 steps (Rodrigues et al., 2016):

(a) Firstly, design of the 3D model by combining porous layers with 0/90 degrees orientation in respect to the x-axis, in which each layer consists of parallel filaments separated by a specific gap (0.65 mm), which is the pore size of the printed part.

(b) Secondly, the 3D model in STL format file is exported to the slicing software (Cura software) where it is sliced into horizontal layers with a defined thickness of 0.2 mm, resulting in a ‘3 slice per layer’ approach. At the end of build step, a g-code file is created, which contains the path/coordinates that can be recognized by the printer to fabricate the 3D model.

(c) Finally, the g-code is sent to the 3D printer which uses a temperature controlled nozzle (diameter 0.4 mm), to extrude the material and deposit the semi-molten material onto a platform in a layer-by-layer process. The filament is moved by two rollers and acts as a piston to drive the semi-molten material. When one layer finished, the platform is lowered and the next layer is deposited. The designed object is fabricated based solely on the precise deposition of thin layers.

In the printing process, parameters such as printing speed and printing temperature need to be desired in optimum to achieve a constant flow rate and consequently a constant filament width with a minimal fabrication time. Figure 2.5 illustrated the manufacturing process for a porous tissue scaffolds using a FDM-3D printing technique (An et al., 2015).

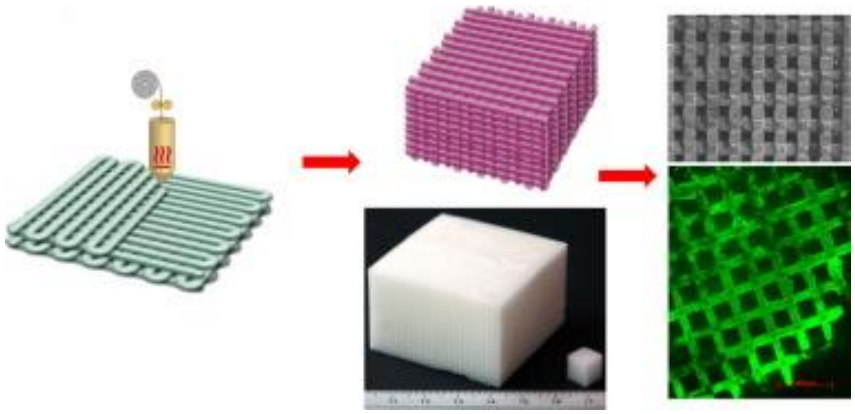


Figure 2.5 Three-dimensional Printing of Tissue Scaffolds (Mohanty et al., 2015)

2.3 Polylactic Acid (PLA) Biomaterial for 3D Printing

Typically, there are four individual groups of natural or synthetic biomaterials are commonly used in the fabrication of scaffolds for tissue engineering depending on the type of tissue regenerated. These materials include metals, ceramics, natural/synthetic polymers, and composites.

Among these materials, polymers remain as the major used material for 3D printing of scaffolds for tissue engineering. Such polymers include synthetic polymer or natural polymer, which both used in forming of hydrogels. Hydrogels are biomaterials that exhibited adjustable mechanical properties, biocompatible and biodegradable. It has the ability to be hydrated while remaining insoluble and thus maintaining their 3D structure. The hydrating properties of hydrogels enable them to mimic those extracellular matrix (ECM) biological environment (Do, Khorsand, Geary, & Salem, 2015).

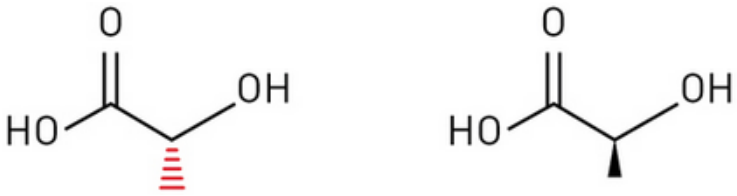
For natural polymer used for scaffold fabrication, it was referred to biological materials such as collagen, proteoglycans, chitosan, etc. These materials are biologically active, and typically promote excellent cell growth. However, the natural polymer made scaffolds generally have poor mechanical properties, which limits their use in load bearing orthopedic applications. The poor in strength also make it not suitable for used in 3D printing technique that involves the filament stretching process (Mohanty et al., 2015).

Among the types of synthetic polymer biomaterials available in the market today, polylactic acid (PLA) is the most extensively researched and utilized biodegradable and renewable feedstock materials used for 3D printing technology. It is

the commercial thermoplastic used in fabrication biodegradable medical devices, especially soft tissue scaffold. Based on research statistic on the PLA market size in 2016, the global PLA market is expected to achieve a forecast of USD 2,169.6 million or production quantity of over 800,000 tonnes by year 2020. The increasing demand in 3D printed scaffolds in this recent year to solve the problem tissue/organ transplantation is expected to further drive market of PLA over the next few years (Grand View Research, 2016).

2.3.1 Chemical Structure of Polylactic Acid (PLA)

Polylactic acid (PLA) is a chemical that belongs to the family of aliphatic polyesters, and it made up by the lactic acid (2-hydroxy propionic acid, LA) building block. Since it is a chiral molecule, the building block can exist in two optically active enantiomers, $_{D}$ or $_{L}$-lactic acid (Garlotta, 2002). Figures 2.6 and Figure 2.7 shows the monomer and skeletal structure of polylactic acid respectively.



(a) D-lactic acid **(b) L-lactic acid**

Figure 2.6 Monomer structure of Poly(lactic acid)

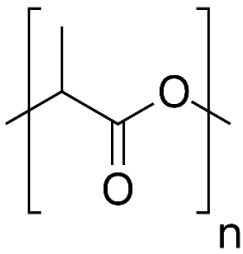


Figure 2.7 Skeletal structure of Poly(lactic acid) (Gupta and Kumar, 2007)

2.3.2 Synthesis of Polylactic Acid (PLA)

The monomer of polylactic acid (PLA), called lactic acid (LA) can be produced from fermentation of renewable natural sources (mainly starch and sugar feedstock, corn and rice), or chemical synthesis. However, in current, majority of lactic acid production is based on the fermentation route. Figure 2.8 shows the whole process for fermentation.

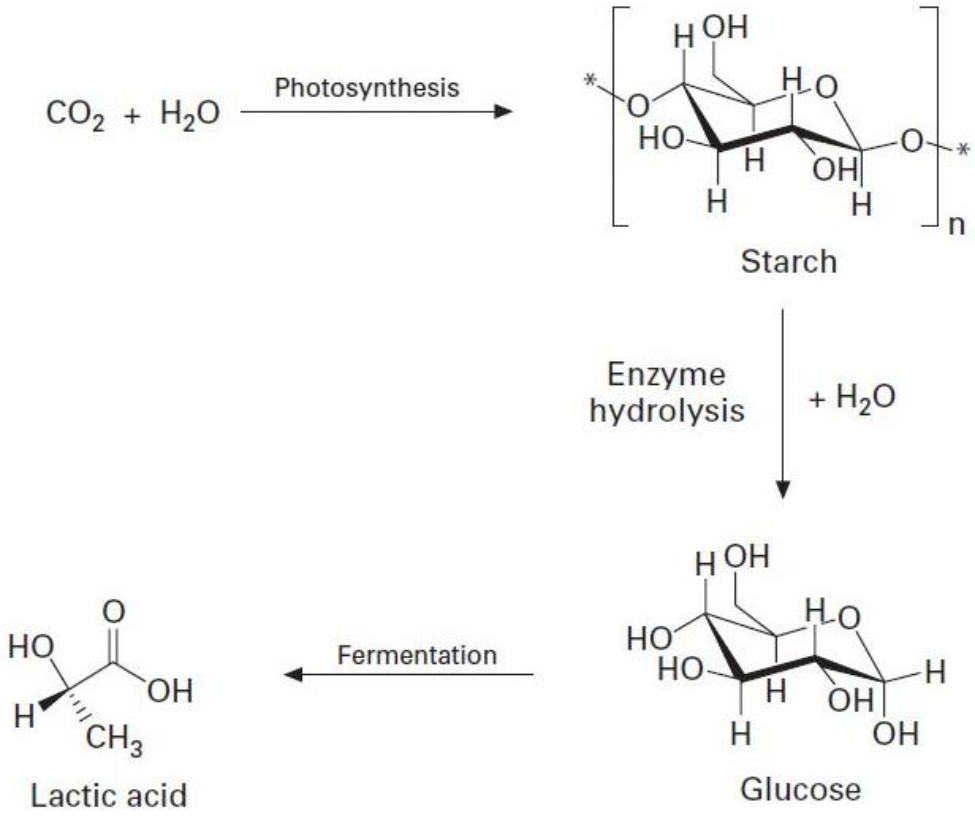


Figure 2.8 Fermentation Route of Lactic Acid (LA)

In the history of polymerization of lactic acid, polylactic acid (PLA) was synthesized in 1982 by Carothers (at Du Pont). However, he was only able to produce a low molecular weight (M_w) PLA by heating the lactic acid under vacuum while removing the condensed water as the by-product of polymerization. The presence of water molecule tends to degrade the forming polymer chain to the point that only very

low molecular weight PLA are obtained. The low molecular weight PLA is very low in strength properties which limits their application (Farah, Anderson, & Langer, 2016).

The problem at that time was the low molecular weight of the PLA; and, finally, by introducing ring-opening polymerization of the lactic acid, a high molecular weight PLA was synthesized. In this mechanism, the cyclic lactide monomer is make through oligomerized the lactic acid and then catalytically dimerized it. Through this polymerization with the employed of a stannous actuate catalyst, a high molecular weight PLA produced. In this mechanism, no any additional water molecule generates at as by-product, hence a wide range of molecular weight is accessible (Farah et al., 2016). Figure 2.9 shows the ring-opening mechanism of lactic acid.

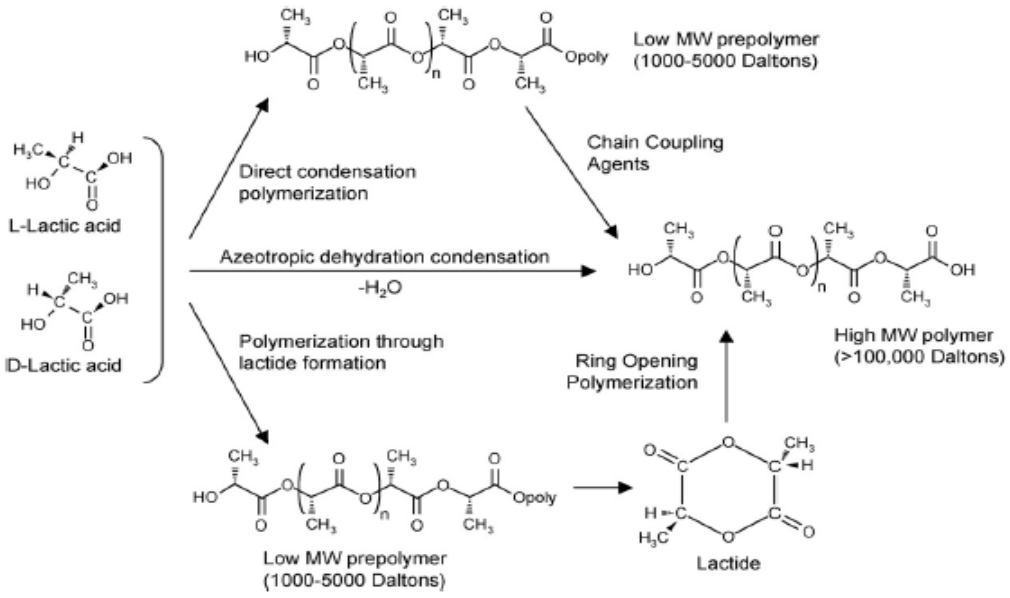


Figure 2.9 Synthesis of PLA from L- and D- Lactic acids (Lim et al., 2008)

2.3.3 Properties of Polylactic Acid (PLA)

Properties of polylactic acid (PLA) was dependent on several factors e.g. isomers content, processing temperature, annealing time and molecular weight. In term of component isomers, PLA polymers with L-isomer content greater than ~90% tend to be crystalline while those with lower L-isomer content are amorphous. Crystallinity influences many polymer properties e.g. hardness, modulus, tensile strength, stiffness, crease and melting points. The melting, glass transition temperature, and crystallinity decrease with decreasing L-isomer content (L. T. Lim, Auras, & Rubino, 2008).

Generally, processing characteristic of polylactic acid (PLA) also dependent on isomer contents. Usually, PLA product which require heat-resistance properties can be injection molding using PLA resins of high percentage of L-isomer, or means highly crystalline PLA. Alternatively, nucleating agents may be added to promote the crystallinity under relatively short molding cycles. In contrast, PLA resins of higher D-isomer (low crystallinity level) would be more suitable for thermoformed, extruded, and blow molded products, since they are more easily processed when the crystallinity is low due to low processing temperature (L. T. Lim et al., 2008).

Physical characteristics such as density, heat capacity, and rheological properties of polylactic acid (PLA) are dependent on its crystallinity level. The density for crystalline and amorphous solid PLA are 1.36 g/cm^3 and 1.25 g/cm^3 respectively. For thermal properties, a high crystallinity PLA result in high melting temperature (~150-160^{o}C). In contrast, a high amorphous PLA result in high glass transition temperature (~55-70^{o}C). While for rheological properties, PLA exhibited Newtonian behaviour at the low shear rates (<10 s^{-1}) whereas it exhibited non-Newtonian behaviour (shear