OPTIMIZATION OF BIOACTIVE POROUS POLY (LACTIC ACID) SCAFFOLD FOR BIOMEDICAL MATERIALS

This report is submitted in accordance with requirement of the Universiti Teknikal Malaysia Melaka (UTeM) for Bachelor Degree of Manufacturing Engineering (Engineering Materials) (Hons.).

by

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2017
DECLARATION

I hereby, declared this report entitled “Optimization of Bio Active Porous Poly (Lactic Acid) Scaffold for Biomedical Materials” is the result of my own research except as cited in references.

Signature : …………………………………………
Author’s Name : Advin Phang Kok Seng
Date : 19th June 2017
APPROVAL

This report is submitted to the Faculty of Manufacturing Engineering of Universiti Teknikal Malaysia Melaka as a partial fulfilment of the requirement for Bachelor of Manufacturing Engineering (Engineering Materials) (Hons). The member of the supervisory committee is as follow:

……………………...........................................................

(Supervisor)

(DR. ZALEHA BINTI MUSTAFA)
ABSTRAK

Perancah poros yang terdiri daripada poli (laktik) asid (PLA) dan pengisi bioaktif kalsium fosfat adalah direka dengan menggunakan gabungan proses acuan pelarut, pengacuan mampatan panas dan proses larut lesap garam. Pengoptimuman peratusan berat porogen ditentukan berdasarkan morfologi liang, saiz liang, bilangan keliangan serta kekuatan mampatan dan ia dianalisis dengan menggunakan mikroskop imbasan elektron, analisis keliangan, analisis haba, Fourier spektroskopi inframerah dan ujian kekuatan mampatan. Objektif kajian ini adalah untuk menilai ciri liang, mengoptimumkan kekuatan mampatan menggunakan peratusan berat porogen yang berbeza (wt.%), dan membandingkan kesan pengisi kalsium fosfat pada kekuatan mampatan perancah poros berdasarkan PLA. Pada 80 % daripada peratusan berat porogen, perancah mempamerkan modulus mampatan 116.39 ± 10.6 MPa, dengan keliangan 75.39 % dan ia mempamerkan struktur liang saling berhubung dan terbuka. Dengan penambahan pengisi bioaktif, modulus mampatan telah meningkat dengan ketara kepada 133.86 ± 9.71 MPa. Modulus mampatan perancah poros PLA terkemuka dan setanding dengan sifat tulang trabekular manusia (50-250 MPa). Kalsium fosfat pengisi bukan sahaja dapat meningkatkan kekuatan mekanikal tetapi ia juga berupaya untuk mengalakkan pertumbuhan semula tulang. Selain itu, kehadiran porogen juga berupaya untuk berfungsi sebagai ejen penukleusan yang meningkatkan suhu peralihan kaca Tg dan mengurangkan penghabluran PLA. Ini adalah disebabkan oleh keupayaan porogen untuk merintangi pergerakan rantaian daripada molekul PLA.
ABSTRACT

Porous scaffold comprising of poly (lactic acid) PLA and bioactive filler calcium phosphate are fabricated by the combination of solvent casting, hot compression moulding and salt leaching process. The optimization of the porogen loadings is determined based on the pore morphology, pore size, porosity as well as the compressive behavior and it is carried out by using Scanning Electron Microscope (SEM), porosity analysis, Differential Scanning Calorimetry (DSC), Fourier Transform Infrared Spectroscopy (FTIR) and compression testing. The objectives of this research are to evaluate the pore characteristic, to optimize the compressive behavior using different porogen loadings (wt.%) and to compare the effect of the calcium phosphate filler on the compressive behavior of the porous PLA scaffold. At 80 % of porogen loadings, the scaffolds exhibit a compressive modulus of 116.39 ±10.6 MPa, with a porosity of 75.39 % and it exhibit interconnected open pore structure. With the addition of the bioactive filler, the compressive modulus has increased significantly to 133.86 ± 9.71 MPa. The prominent compressive modulus of the porous PLA scaffold is comparable with the human trabecular bone (50-250 MPa). The calcium phosphate filler not only improved the mechanical strength but also induced bioactivity to allow bone regeneration. Besides that, with the presence of the porogen, it has act as a nucleating agent which increase the glass transition temperature $T_g$ and decrease the crystallinity of the PLA. This is mainly due to the porogen has impedes the chain mobility of the PLA.
DEDICATION

Dedicated to
my beloved father, Phang Wei Khean
my appreciated mother, Wong Mei Kuan
and my adored siblings Edward Phang Jun Seng
for giving me moral support, cooperation, encouragement and also understanding.
ACKNOWLEDGEMENT

First of all, I would like to express my deepest gratitude to my beloved supervisor, Dr. Zaleha binti Mustafa for her kind patronage, loving inspiration, valuable information and timely guidance, which helped me in completing the Final Year Project on time and fulfill the requirements.

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Last but not least, I would like to show my deepest appreciation to my family members for their moral support, understanding and encouragement throughout this project.
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LIST OF ABBREVIATIONS

\(\mu\)CT - Micro-computed Tomography
3D - Three Dimensional
ACP - Amorphous Calcium Phosphate
\(\text{Al}_2\text{O}_3\) - Aluminum Oxide
AM - Additive Manufacturing
\text{CaCO}_3 - Calcium Carbonate
CAD - Computer-Aided Design
CaP - Calcium Phosphate
CF - Chitin fibres
\text{CO}_2 - Carbon Dioxide
FDM - Fused Deposition Modelling
\text{H}_2\text{O} - Water
HA - Hydroxyapatite
LDM - Low Temperature Deposition Manufacturing
\text{Mg-NPs} - Magnesium Hydroxide nanoparticles
\text{NaHCO}_3 - Sodium Bicarbonate
\text{NaCl} - Sodium Chloride
nCaP - Nano Calcium Phosphate
nHACP - Nanohydroxyapatite/Collagen/poly (lactic acid)
PCL - Poly (\(\varepsilon\)-caprolactone)
PDLA - Poly (D-lactide)
PDLLA - Poly (DL-lactide)
PGA - Polyglycolide
pH - Potential of Hydrogen
PHBV - Poly (hydroxybutyrate-cohydroxyvalerate)
PLA - Poly (lactic acid)
PLLLA - Poly (L-lactide)
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<tr>
<td>PUR</td>
<td>Poly (ester urethane)</td>
</tr>
<tr>
<td>PUs</td>
<td>Polyurethanes</td>
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<tr>
<td>RP</td>
<td>Rapid Prototyping</td>
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<td>SEM</td>
<td>Scanning Electron Microscopy</td>
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<td>TCP</td>
<td>Tricalcium Phosphate</td>
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<td>ZrO₂</td>
<td>Zirconium Oxide</td>
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LIST OF SYMBOLS

wt.% - Weight Percentage
vol.% - Volume Percentage
MPa - Mega Pascal
GPa - Giga Pascal
T_m - Melting Temperature
T_g - Glass Transition Temperature
T_c - Cold Crystallization Temperature
°C - Degree Celsius
J/g - Joule per Gram
mm - Millimetre
µm - Micrometre
g/cm^3 - Gram per Cubic Centimeter
g/mol - Molar Mass
X_c - Crystallinity
CHAPTER 1
INTRODUCTION

1.1 Background Study

Over the past decades, researchers have made some breakthrough in the field of bone tissue engineering in the aspect of new materials, improve the processing techniques, and enhance their application (Li et al., 2013). The most notable growth that has been achieved are in the development of porous scaffold materials for the bone healing and structural support with optimum osteogenesis abilities. Bone scaffold serves as a template during bone tissue regeneration. Technologies today does not only allow the fabrication of the porous scaffold with controlled porosity but also enhanced properties with it. Natural human bone has a special combination of both mechanical properties and architectural design from macro to nanoscale dimensions as shown in Figure 1.1. Thus, in order to tailor the desired properties such as porosity, strength, toughness in combination with degradable properties of the chosen materials evolving into the nanostructures to imitate the nature of the bone’s nanocomposite architecture in necessary. It is also preferable that the selected materials must give similar feedback as human bone, biocompatible with the host tissue. The scaffold must aid adhesion, proliferation, and secretory activities of the cell. They also need be compatible in term of mechanical properties with the human bone (Yang et al., 2001).
Poly lactic acid (PLA) is a trending aliphatic polyester that has been used as drug delivery, surgery and tissue engineering due to their outstanding properties in comparison to the other degradable polymer (Guarino et al., 2008; Silva et al., 2016). PLA is a plant-derived polymer which can be frequently obtained from renewable resources such as corn, sugar cane and starch. PLA is also an environmental friendly polymer because when it degrades, it will produce carbon dioxide (CO$_2$) and water (H$_2$O).

However, as PLA is not a bioactive material, incorporation of calcium phosphate (CaP) materials such as hydroxyapatite (HA) and tricalcium phosphate (TCP) are often use to induce the bone cell formation. HA has a crystalline phase and it can be found in the component of bone which has been frequently used for bone tissue engineering (Guda et al., 2011; Jun et al., 2013). One of the main reason is because HA acquires the osteoconductive properties which allows bone growth, thus encourage fracture bone cell to regenerate during the healing process.
1.2 Problem Statement

Tissue and organ failures in human which caused by infections, injuries and defects are one of the most catastrophic and expensive encounters in human health care. The main problem for the patients is the lack of organ and tissue donor. Therefore, various types of surgical strategies has been discovered to fix these issues by introducing artificial substitutes and non-living processed tissues (Rai et al., 2012). However, such substitutes do not really solve the deficiency of the bone or the functions of the host tissues. For instances, lack of biocompatibility, non-bioactive material, vascularization of bone and bone remodelling (Lafage-Proust et al., 2015). Hence, bioactive, biodegradable and biocompatible materials have been brought into the field of tissue engineering for a greater exploration (Puppi et al., 2010).

Bone grafting is another surgical process which can be used to solve problems with the bones and joints. It considers two types which are autograft and allograft. Autograft is the bone or tissue that will be transplanted from one site to another site in the same patient while allograft is from the donor to the patient. Autograft is a very promising treatment but it has a few problems such as high cost, increase morbidity and the risk of getting an infection. Likewise, allografts also have a setback which is the risk of rejecting by the patient’s immune system which might have a great chance of suffering from an infection from the donor to the patients (O’Brien, 2011). On the other hand, the main purpose of tissue engineering is to create a biological substitute that are able to heal the damaged tissues but not replacing it. Therefore, porous scaffold is introduced in the field of tissue engineering.

An ideal porous scaffold must have an interconnected pores and mechanical properties that are close to the bone. It is important to fulfil these criteria but there is a common challenge in fabricating the porous scaffold which is to control the level of compressive strength and stiffness while maintaining the open pore structures with the optimum pore characteristics (Porter et al., 2014). Therefore, developing a highly porous scaffold must have sufficient mechanical strength to receive high loads and at the same time assist in the tissue regeneration.
The current development in the field of porous scaffold tissue engineering has two different types of fabrication techniques which are additive manufacturing (AM) and the conventional fabrication method. Both of these methods have their own pros and cons for the researcher to work with in order to fabricate biological porous scaffold. Regarding the additive manufacturing (AM), it involves three dimensional (3D) printing, fused deposition modelling (FDM), selective laser sintering and stereolithography process. These processes can control the size and interconnected pores and able to produce complex shapes from a computer-aided design (CAD) model. The limitation of the AM which is the resolution is controlled by the jet size, so it will make it very hard to produce scaffold with fine microstructures (Liu & Ma, 2004). Another problem of AM will be the shortage of advanced biomaterials that can exhibit good behaviour with the native bone tissues of the human body. On the other hand, for the conventional fabrication, it involves salt leaching, freeze casting and electrospinning. Salt leaching process, it is a very simple approach but it is limited in the control of pore shape and connectivity. The pore shape can only rely on the particle size of the salt. It is also difficult to leach out salt particles in the interior of a polymer matrix and this has restricted it to fabricate thicker porous scaffold (O'Brien et al., 2014). As normally in salt leaching process, the polymer matrix that been used is degradable polymer and the scaffold were formed by direct casting method, thus it was not fully dense and contribute to low mechanical properties and lack of apatite inducing properties. Thus, this study will explore the possibility to produce high initial strength of porous scaffold by combining normal salt leaching process with high pressure moulding to produce denser scaffold and improve their pore formation. Nano calcium phosphate will be used as nano filler to further enhance their mechanical properties as well as increase the apatite formation ability.
1.3 Objectives

1. To evaluate the pores characteristic of the scaffold using morphological analysis.

2. To optimize the compressive strength of the porous PLA scaffold using different porogen loadings (wt.%).

3. To compare the effect of calcium phosphate filler on to the compressive strength of the porous PLA scaffold.

1.4 Scope

The scope of this study will focus on the fabrication of bone scaffold by using solvent casting, hot compression moulding and the salt leaching process to produce porous scaffold. The biodegradable PLA matrix will be prepared using different loading of the porogen (80, 90, 93, 95 wt. %) to optimize their porosity and pore connectivity (open pore) as well as their compressive strength. CaP will be added as bioactive filler to enhance the bioactive and the strength of the scaffold. The porous scaffold will be characterized by using thermal analysis, porosity analysis, chemical analysis, compression testing and morphological evaluation.
CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

The development of the reconstruction operating method in orthopaedics which related to damage, tumour, deterioration and an aging population has trigger a bloom, mainly related to the clinical growth and also the evolution of bone scaffold tissue engineering. In the old days, porous scaffolds are fabricated from synthetic metals, polymers, ceramics and composite biomaterials. These materials do not take into account of the native structure or the properties of cells and the nature of the tissues. These synthetic scaffolds have also merge deficiently together among the body cells and the surrounding host tissue, thereby lead to undesirable surgical which results unfavourable properties like mechanical mismatch and poor surface environment. Nowadays, the biodegradability, biology of the cell, biomaterials and mechanical properties, in which this features are considered for the ideal bone scaffold (Wu et al., 2014).

2.2 Bone

Bone is an essential, active connective tissue that sustain the shape of the body, support and protect the major organs, and it enables mobility for the locomotion for the body (Rouhi, 2012). To sustain all these functions, the bone should acquire high stiffness and toughness that are enough to carry the whole body’s weight and prevent fracture. Bone is also an active tissue that induce a various types of bone cells for the proliferation and vascularization of network of cells. In short, it is vital for both biomechanically and metabolically for the body.
2.2.1 Bone Structure

Bone structure is mainly made up of interconnected pores. Human consist of two bone structure which are cortical and cancellous bone. The hard outer layer of the bone is cortical bone while the bone that are situated in the central part in the spine and the tip of the long bones of the vertebral bones are cancellous bone. The main dissimilarity of the cortical and cancellous bone are cortical bone is having less than 10 vol% porosity while cancellous having 50-90 vol% porosity (Bose et al., 2013). The interconnection and the size of the pore are important in the bones because it will affect the diffusion of oxygen and nutrients, cell attachments and the new cell formation. From the aspect of the mechanical strength, the strength of the bone tissue for cortical and cancellous bone has a range of 1-12 MPa and 150-200 MPa respectively (Demers et al., 2002; Manassero et al., 2016).

The skeleton can be split into two subdivisions which are axial and appendicular as shown in Table 2.1. These two subdivision of the skeleton is divided into axial and appendicular parts according to their functions. The process of bone remodelling and cellular signals from coupling signals from the cortical and trabecular bone is shown in Figure 2.1.

Table 2.1 Subdivision of the Skeleton (Bronner & Worrell, 1999)

<table>
<thead>
<tr>
<th>Feature</th>
<th>Axial</th>
<th>Appendicular</th>
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<tr>
<td>Major bone tissue</td>
<td>Cancellous</td>
<td>Cortical</td>
</tr>
<tr>
<td>Adjacent soft tissues</td>
<td>Viscera</td>
<td>Muscle</td>
</tr>
<tr>
<td>Cortices</td>
<td>Thin</td>
<td>Thick</td>
</tr>
<tr>
<td>Marrow</td>
<td>Hematopoietic</td>
<td>Fatty</td>
</tr>
<tr>
<td>Turnover</td>
<td>High</td>
<td>Low</td>
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<table>
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<tr>
<th>Main functions</th>
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<tbody>
<tr>
<td>Cortical</td>
<td>Mechanical</td>
<td>Mechanical</td>
</tr>
<tr>
<td>Cancellous (^a)</td>
<td>Metabolic</td>
<td>Mechanical</td>
</tr>
</tbody>
</table>

\(^a\)Metabolic activity such as uptake and release of calcium occurs in ll cancellous bone in red marrow sites throughout the skeleton.
2.2.2 Woven and Lamellar Bone

Woven bone is randomly oriented with collagen fibres, osteoblasts and osteoprogenitor cells in it. Woven bone is also mainly found during the development of foetus and callus which is formed during the repairmen of fracture bone part. In most of the bone, woven bone contains osteocytes and blood vessels. In the alveolar socket of the adult cavity can often found areas of woven bone.

Lamellar bone has a more specific arrangement and it is form much more slowly compared to woven bone. The mature form of the adult bone is mainly lamellar bone. Besides that, the rate of deposition is slow for the well-arranged of collagen fibres (Currey, 2014). The lamellar or Harvasian bone also form an open structure of the cancellous bone structure. Both structural diagram of woven and lamellar bone is shown in Figure 2.2.