DEVELOPMENT OF POLY(LACTIC ACID) MICROSPHERES/GELATIN COATED BETA-TRICALCIUM PHOSPHATE SCAFFOLD FOR BONE TISSUE ENGINEERING APPLICATION

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LIST OF ABBREVIATIONS

API	Active pharmaceutical ingredients
BCA	Bicinchoninic acid assay
BSA	Bovine serum albumin
β-TCP	Beta-tricalcium phosphate
$CaCO_3$	Calcium carbonate
$CaSO_4$	Calcium sulfate
$CaAl_2O_4$	Calcium aluminium oxide
Ca_3PO_4	Tricalcium phosphate
Ca^{2+}	Calcium ions
DCM	Dichloromethane
ECM	Extracellular matrix
E.coli	Escherichiacoli
FDA	Food and drug administration
FTIR	Fourier transform infared spectroscopy
GA	Glutaraldehyde
HA	Hydroxyapatite
He	Helium
NaCl	Sodium chloride
o/w	Oil-in-water
PBS	Phosphate buffered saline
PEG	Poly(ethylene glycol)
PEI	Poly(ethyleneimine) solution
PHA	Polyhydroxyalkanoates

PHB	Polyhydroxybutyrate
PLA	Poly(lactic acid)
PLGA	Poly(lactide-co-glycolides)
PO_4^{3-}	Phosphate ions
PVA	Poly(vinyl alcohol)
SBF	Stimulated body fluid
SDS	Sodium dodecyl sulfate
SEM	Scanning electron microscopy
SiC	Silicon carbide
Si_3H_4	Trisilicontetrahydride
SPGE	Sorbitol polyglcidyl ether
T_g	Glass transition temperature
UV-Vis	UV-visible spectroscopy
w/o	Water-in-oil
w/o/w	Water-in-oil-in-water
3D	Three-dimensional
α-TCP	Alpha-tricalcium phosphate

LIST OF SYMBOLS

%	Percentage
℃	Celsius
℃/min	Celsius/minute
cm^3	Cubic centimetre
CFU/ml	Colony-forming unit/milimeter
g/cm^3	Gram/cubic centimetre
g/mol	Gram/mole
GPa	Gigapascal
H	Hours
hPa	Hectopascal
kV	Kilo Volt
min	Minutes
mm	Milimeter
ml	Mililitre
mg	Miligram
mg/ml	Miligam/mililitre
mPa s	Milipascal seconds
M	Molar
MPa	Megapascal
µl	Microlitre
µm	Micrometer
nm	Nanometer
rpm	Rotation per minute

s	Seconds
v/v	Volume/volume
w/v	Weight/volume

PEMBANGUNAN PERANCAH BETA-TRIKALSIUM FOSFAT BERSALUT MIKROSFERA POLI(LAKTIK ASID)/GELATIN UNTUK APLIKASI KEJURUTERAAN TISU TULANG

ABSTRAK

Struktur bersambungan liang tiga dimensi dan kekuatan mekanikal yang ideal adalah penting bagi perancah biologi dalam aplikasi kejuruteraan tisu tulang. Tujuan utama kajian ini adalah untuk menghasilkan perancah yang pelbagai fungsi bagi aplikasi kejuruteraan tisu tulang. Dalam kajian ini, kaedah pengacuan gel digunakan untuk menghasilkan perancah beta-trikalsium fosfat (β-TCP) berliang. Perancah β-TCP disalut dengan gelatin sambung silang bersama 1% glutaraldehyde (GA) menunjukkan kekuatan mampatan optimun di mana ia meningkatkan 139% kekuatan mampatan berbanding dengan perancah yang tidak disalut. Perancah β-TCP dan perancah β-TCP disalut dengan gelatin sambung silang menunjukkan sifat bioaktif dengan mengambil 2 minggu untuk pembentukan apatit. Penyalutan gelatin mengurangkan penyerapan protein berbanding dengan β-TCP asli. Dalam peringkat kedua, emulsi pemeruapan pelarut digunakan untuk menyediakan mikrosfera poli(laktik asid) (PLA) yang dimuatkan *doxycycline*. Keputusan menunjukkan bahawa 4% NaCl dalam fasa akueus luaran adalah cara yang paling berkesan untuk meningkatkan kecekapan pengkapsulan, peningkatan 66% pengkapsulan *doxycycline* dan diameter zon pertumbuhan halangan terbesar E. coli iaitu pada 16.15mm telah dihasilkan. Akhirnya, perancah β-TCP bersalut mikrosfera PLA telah meningkatkan kekuatan mampatan. *Doxycycline* yang terkapsul dilepaskan dengan lebih lambat dan berkekalan berbanding dengan mikrosfera PLA bebas. Ringkasnya, perancah bersalut mikrosfera PLA dihasilkan dalam kajian ini menunjukkan pelbagai fungsi di mana ia merupakan struktur sokongan, bioaktiviti dan fungsi penghantaran dadah terkawal bagi kejuruteraan tisu tulang.

DEVELOPMENT OF POLY(LACTIC ACID) MICROSPHERES/GELATIN COATED BETA-TRICALCIUM PHOSPHATE SCAFFOLD FOR BONE TISSUE ENGINEERING APPLICATION

ABSTRACT

Three-dimensional interconnected porous structure and ideal mechanical strength are essential for biological scaffold in bone tissue engineering application. The main aim of this study is to develop multifunctional scaffold for bone tissue engineering application. In this research, gel casting method was used to produce porous beta-tricalcium phosphate (β-TCP) scaffold. β-TCP scaffold coated with gelatin crosslinked with 1% glutaraldehyde (GA) showed optimum compressive strength where it improved around 139% compressive strength compared to uncoated scaffold. Uncoated β-TCP scaffold and the β-TCP scaffold coated with crosslinked gelatin showed their bioactive properties by taking 2 weeks to form apatite. The gelatin coating had slightly reduced protein-adsorption compared to pure β-TCP. In the second part, emulsion solvent evaporation was used to fabricate doxycycline loaded poly(lactic acid) (PLA) microspheres. Results showed that the 4% NaCl in external aqueous phase was the most efficient way to increase the encapsulation efficiency where 66% of the doxycycline was encapsulated and showed the largest inhibition growth zone diameter of *E. coli* at 16.15mm. Lastly, β-TCP scaffold coated with PLA microspheres had enhanced the compressive strength. The encapsulated doxycycline was released in a slower and sustained manner compared to free PLA microspheres. In short, PLA microspheres coated scaffold developed in the study shows multifunctional as it provides structural support, bioactivity and controlled drug delivery function for bone tissue engineering.

CHAPTER 1

INTRODUCTION

1.1 Research Background

The concept of tissue engineering involves the process in which it creates suitable micro-environment for the survival of cell so that it can help the regeneration of damaged tissues or organs such as bones, cartilage, skin, etc. and consequently enhances the natural healing potential of patients (Singh et al., 2016). It aims to repair injured tissue with the aid of scaffold to guide the geometrical of the new tissue (Williams, 2004). Biomaterial-based scaffold plays an imperative function in bone tissue engineering since it can affect the matrices of tissue formation (Elango et al., 2016). An effective extracellular matrix or scaffold required for tissue regeneration should provide high porosity, interconnected pores, biocompatible, biodegradable, non-cytotoxicity, osteoconductivity and mechanical match (Yang et al., 2001; Elango et al., 2016; Dhandayuthapani et al., 2011).

There are some requirements need to be achieved during the fabrication of scaffold which include mechanical strength, pore size, porosity and interconnectivity. The appropriate porosity and pore size play a significant role since they allow cell migration and proliferation, metabolite and nutrient diffusion and also vascularization (Karageorgiou and Kaplan, 2005). The minimum recommended pore size for a bone substitute is 100μm but subsequent studies have shown better osteogenesis for substitutes with pores greater than 300μm (Hannink and Arts, 2011). In bone tissue engineering, porosity can be categoried into two types which are macroporosity and microporosity, where the pores size is greater than 100μm and less than 10μm, respectively (Fisher et al., 2012). Macroporosity influence the cell

distribution, migration and allows *in-vivo* blood vessel formation to help in the formation of new bone tissue. While, microporosity provides large surface area for protein adsorption and greater surface area for protein adsorption and give rise to high solubility of ionic. Interconnectivity could affect the degree and path of tissue regeneration. Besides, according to Zhou et al. (2016) the large surface area and the roughness of the scaffold are essential factors in cell seeding and fixation.

Bioceramic is the ceramic that use to repair and replace the diseased tissues or organs. Bioceramic has been categorized into three basic types which are bioinert ceramics, bioactive ceramics and bioresorbable creamics. Bioinert ceramics (eg. Alumina (Al_2O_3), Zirconia (ZrO_2)) have the properties of high chemical stability *in vivo* and high mechanical strength. Bioactive ceramic (e.g. bioactive glass, glass ceramic) have the characteristic of osteo-conduction and are capable to form chemical bond with living tissue (Poitout, 2016; Thamaraiselvi and Rajeswari, 2004). Bioresorbable ceramics such as calcium phosphate can actively take part in the metabolic processes of an organism with the predictable results (Thamaraiselvi and Rajeswari, 2004).

The most commonly used calcium phosphate ceramics family are hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) (Thamaraiselvi and Rajeswari, 2004). These two calcium phosphate-based materials are broadly used as synthetic bone graft. HA can directly form bond to the host bone but are poor in degradable process (Yang et al., 2016). On the other hand, β-TCP is a bioresorbable material that can totally supplant the autogenous bone tissues because of its almost similar chemical to biological apatite in human being hard tissues (Xie et al., 2016).

The mechanical properties of the scaffold are very vital in handling prior to and throughout the surgical implantation. Besides, it also important in the repair of load bearing bone, in which it will be subjected to mechanical loads *in-vivo* while tissue is regenerated. However, the main disadvantages of the bioceramic scaffold are very brittle and low strength (Motealleh et al., 2016). For the purpose of improving the mechanical properties of bioceramic scaffolds such as fracture strength and crack resistance, polymer coatings are found to be one of the approaches to strengthen and toughened the scaffold by the activation of a micron scale crack-bridging mechanism (Li et al., 2014). The polymeric coating film will undergo continuous deformation and form thin fibrils which are able to bridge the cracks edges, this crack-bridging mechanism are opposing to the crack opening process and prevent the catastrophic fracture of the entire structure of the scaffold. Consequently, it can increase the fracture strength and crack resistance of the bioceramic scaffolds (Bertolla et al., 2014).

In this context, to fabricate scaffolds with excellent properties, it can be done by coating the ceramic with natural polymers such as carrageenan, gelatin, alginate, silk, collagen, chitosan and so on (Govindan et al., 2015). By coating the ceramic based scaffold with natural polymers, the compressive strength can be enhanced and suitable as in-situ carriers of therapeutic bioactive molecules such as antibiotics and growth factors. The bioceramic coated with natural polymer commonly shows the improvement in the aspects of *in-vitro* and *in-vivo* biocompatibility, which indicates that this approach are promising for bone tissue engineering applications (Lozano et al., 2014; Yao et al., 2014).

Despite of sufficient mechanical properties, other requirement such as biocompatibility must be fulfilled. With the purpose of improving the biocompatibility, osteoconductivity and bioactivity of the polymer, β-TCP powder was hybridized with the polymer (Kim et al., 2004). Gelatin is used in this research because it can circumvent the immunogenicity issue, pathogen transmission associated with collagen, good cell viability and low cost (Bakhtiari et al., 2010).

In order to promote bone regeneration, there are few efforts have been made to incorporate growth factors and proteins within the scaffolds to make it osteoinductive so that it can function as support as well as drug delivery system by stimulating cell adhesion, proliferation and differentiation (Mourino and Boccaccini, 2009). Microspheres have been used as drug carriers in applications such as medical and biomedical. Polymer microspheres play vital roles in medication delivery with controlled rate to targeted organs or tissues. Release of medication from microsphere is through drug leaching from the polymer or degradation of the polymer matrix (Freiberg and Zhu, 2004). Therefore, microspheres embedded scaffolds are expected to provide a more promising potential for bone tissue engineering applications.

1.2 Problem Statements

The combination of structural scaffolds made by bioceramic scaffold and biopolymer coatings can be conveniently used to add a drug delivery function to the scaffold (Kim et al., 2005). In this strategy, the drug was encapsulated within the polymer microspheres, which uniformly coats the ceramic scaffold. The successful poly(lactic acid) (PLA) microspheres coating on the β-TCP scaffold can be achieved using an optimized coating methods. The goal is to deliver the drug at controlled rate

while the rigid inorganic scaffold maintains the structural integrity of construct, being thus of high interest for bone tissue engineering.

One of the approaches to improve the mechanical strength of porous β-TCP scaffold is by polymer coating (Asaad et al., 2016). Gelatin was used to coat the surface of the scaffold as it readily available, low cost, biocompatible and good cell adhesion (Elzoghby, 2013). Since gelatin is soluble in aqueous solution, gelatin materials for biomedical applications must be crosslinked, which improves the mechanical stability of the biopolymer. Among the chemical crosslinking agents, glutaraldehyde (GA) is by far the most widely used, due to its high efficiency of collagenous materials stabilization. The success of thousands of bioprosthetic implants demonstrated that GA crosslinking has been clinically acceptable and has many merits in spite of the reports on its cytotoxicity (Jayakrishnan and Jameela, 1996). Furthermore, GA crosslinked gelatin microspheres have been successfully employed as microcarriers for the growth and propagation of fibroblasts and endothelial cells (Wissemann and Jacobson, 1985). The biocompatibility of GA crosslinked collagenous materials can be improved by lowering the concentration of GA solutions, as reported for anionic collagenous membranes (Goissis et al., 1999). Hence, gelatin coating crosslinked with low GA concentration was studied to optimize the mechanical strength of β-TCP scaffold.

There exists the challenge to develop effectual drug delivery PLA microspheres. The low hydrophilic drugs encapsulation efficiency in polymeric microspheres is the major drawback. This is because the hydrophilic drug has low affinity to the hydrophobic polymer therefore it causes the partitioning of weakly associated drugs from organic phases to the external water phase during the process

of PLA microspheres formation (Misra et al., 2009). The approach to improve the drug encapsulation efficiency is by manipulating the formulation variables. In the present study, the encapsulation efficiency of doxycycline loaded PLA microspheres are modified by various aspects such as aqueous/organic phase ratio, polymer concentration, drug amount and sodium chloride (NaCl) concentration in external aqueous phase.

1.3 Objectives

The main aim of the research is to develop poly(lactic acid) microspheres incorporated into gelatin coated beta-tricalcium phosphate scaffold. The objectives of this research are:

i. To investigate effect of different concentration of glutaraldehyde on the gelatin coated beta-tricalcium phosphate scaffold.
ii. To characterize effect of formulation variables on the properties of doxycycline loaded poly(lactic acid) microspheres.
iii. To fabricate doxycycline loaded PLA microspheres/crosslinked gelatin coated beta-tricalcium phosphate scaffold.

1.4 Scope of Study

The present study focuses on the fabrication of gelatin coated β-TCP scaffold by studying the effect of glutaraldehyde (crosslinker) concentration on mechanical properties of scaffold. The concentration of glutaraldehyde is varied with 0%, 0.25%, 1% and 1.5%. Besides, uncoated β-TCP scaffold is also prepared as a control purpose and the effect of gelatin coating on bioactivity properties and protein adsorption of scaffold were studied. The second phase of the study concentrates on

the effect of formulation variables on the properties of doxycycline loaded poly(lactic acid) microspheres. The formulation variables include the volume ratio of organic to aqueous phase, polymer concentration, drug amount and sodium chloride concentration in external aqueous phase. This study is continued by using different coating methods to incorporate microspheres onto the gelatin coated scaffold wall such as agitation, ultrasonic and spray method. The effect of different coating methods on the dispersion and attachment of microspheres onto the scaffold was studied. Besides, the study was also concentrates on the properties of the doxycycline loaded PLA microspheres/gelatin coated β-TCP scaffold while gelatin coated β-TCP scaffold was used as control purpose.

1.5 Dissertation Overview

Chapter 1 introduces the idea of scaffold which is important in bone tissue engineering applications. This chapter also includes some of the problem statements for scaffolds and microspheres. The objectives of this research are described in this chapter.

Chapter 2 consists of literature review on the recent progress made in scaffold and microspheres which include background and classification.

Chapter 3 describes the overall research flowchart in the present study, followed by materials used in the experiment and experimental procedures. The characterization methods are also discussed in this chapter.

Chapter 4 reports the result and discussion of the research. This chapter is divided into three parts. The first part discussing the effect of different concentration of glutaraldehyde on the gelatin coated beta-tricalcium phosphate scaffold. The

second parts describes the effect of formulation variables on the properties of doxycycline loaded poly(lactic acid) microspheres. Lastly, the incorporating of microspheres onto the scaffold by using various coating methods is presented and the properties of gelatin coated β-TCP scaffold with and without doxycycline loaded PLA microspheres are compared.

Chapter 5 presents the conclusions from this research and also some recommendations for future studies in this related field.

CHAPTER 2

LITERATURE REVIEW

2.1 Tissue Engineering Concept

The failure of organ or tissue to function causes major health problem of mankind. Tissue engineering is growing rapidly and gain high public noticeable in interdisciplinary field as important potential alternative therapeutic (Devices, 2000). There are four important components of tissue engineering which include cells, scaffolds, signaling molecules and bioreactors. The isolated cell is required to synthesize new tissue; scaffolds provide places for cell adhesion, proliferation and differentiation; signaling molecules include proteins and growth factor which help in tissue regeneration by stimulating cell while bioreactors offer an appropriate biological environment for cell culture (El-Sherbiny and Yacoub, 2013; Ikada, 2006; Akter, 2016).

The main purpose of tissue engineering is to restore or regenerate defected tissue through the combination of cell and 3D porous scaffolds (Sobral et al., 2011). Firstly, the selected cells are isolated from the tissues of human body and cells are being cultivated in two-dimensional for their subsequent growth. Porous three-dimensional scaffolds serve as a template for cell seeding while at the same time growth factors, small molecules and nanoparticles are loaded to biophysically stimulate the cells. The cell seeded scaffolds undergoes further *in-vitro* cultivation in bioreactor to produce functional tissues. Once the functional tissues are successful synthesized then it is transplanted into the injured site of human body. This general concept of tissue engineering is illustrated in Figure 2.1 (O'Brien, 2011; Dvir et al., 2010).

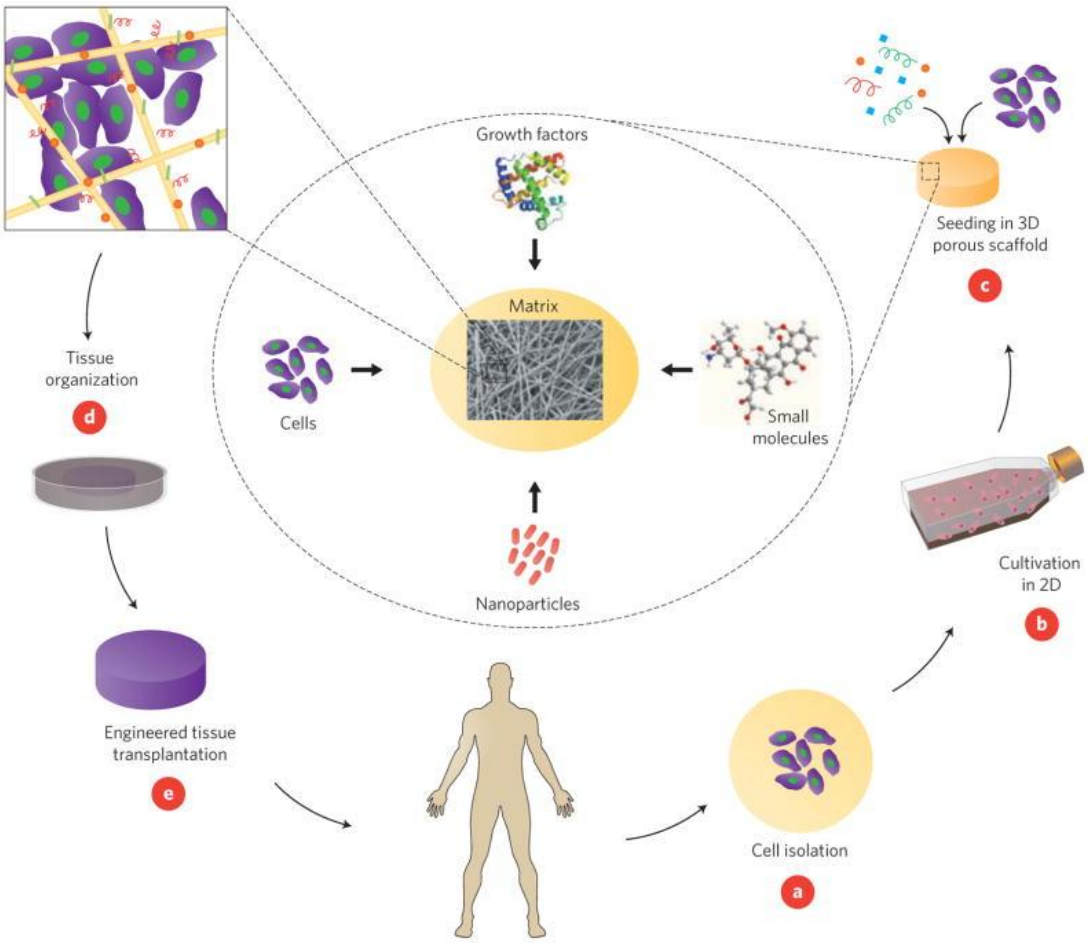

Figure 2.1 A general concept of tissue engineering (Dvir et al., 2010)

2.2 Bone Tissue Engineering

Bone disorder has become remarkably concern as it showed a greatly increasing trend due to aging of population, obesity, trauma, bone disease, tumor resection and lack of exercise (Bose et al., 2012; Amini et al., 2012; Baino and Vitale-Brovarone, 2011). The standard conventional clinical treatment for bone defects includes autograft and allograft. Autograft has excellent osteoconduction, osteoinduction and osteogenesis but it has limited availability of material supply and risk of morbidity donor sites (Amini et al., 2012; Chiarello et al., 2013). Allograft is available in sufficient quantity however the shortcomings are potential infection transmission and lack of osteogenetic properties which may cause inferior healing

(Chiarello et al., 2013; Wang and Yeung, 2017). Therefore, bone tissue engineering has been considered as potential alternative solution to the conventional bone grafting surgical method in which it possess better mechanical properties, unlimited supply and disease transmission can be avoided (Amini et al., 2012; Burg et al., 2000).

The complex process involved in bone tissue engineering initiated with cell migration, cell recruitment and then cell start to proliferate, differentiate and matrix is formed at the same time as bone is remolded. The major advances of scaffold based approaches used in bone tissue engineering are established along with delivery of growth factor, drug and genes (Bose et al., 2012). Biomimetic porous three dimensional scaffolds are synthesized to mimic the extracellular matrix (ECM) of human bone and also provide structural support for bone regeneration and it will gradually degrade so that the new bone can eventually replace the implanted scaffold (Polo-Corrales et al., 2014; Yu et al., 2015).

2.3 Bone Physiology

Bone is complex living connective tissues in human body that constantly maintain its micro-architecture by remodelling throughout life. It is a part of skeleton system that provides structural support and protection for internal organ such as brain, lungs, heart, spinal cord and so on (Stevens, 2008). Besides, it plays an important role in physiological functions which are haematopoiesis, acid-base balance, mineral reserve and locomotion (Walsh, 2015).

2.3.1 Bone Structure and Composition

Bone is categorized into two types which are cortical bone and cancellous bone (trabecular bone) as shown in Figure 2.2. Cortical bone made up approximately 80% in human bone and it is offers the mechanical stability since it is hard and dense. Cancellous bone usually located at the end of long bones and it has the characteristic of low density and spongy architecture (Willems et al., 2014). The bone is made up of organic and mineral matrix. The organic matrix is mainly occupied by type I collagen for about 95% and the remainder 5% is made up of osteonectin, osteocalcin and osteopontin while mineral matrix is two-thirds of total bone and mainly consists of hydroxyapatite crystals and small amount of other magnesium, fluoride sodium and potassium ions. Organic matrix provides bone with toughness while mineral matrix contributes to bone strength (Walsh, 2015).

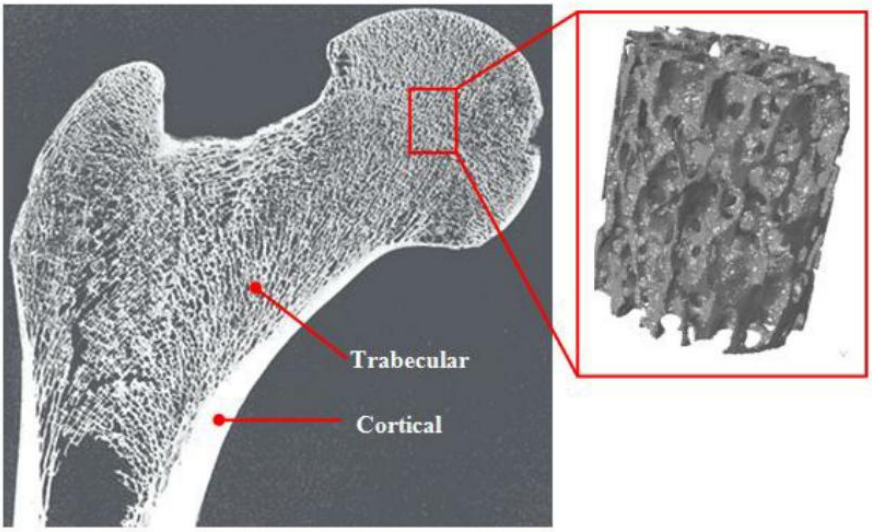

Figure 2.2 Cross section of cortical and cancellous bone at human femur region (Willems et al., 2014)

Figure 2.3 shows five levels of hierarchical architecture of bone. The mechanical properties of bone are closely related to the components and structure of the bones. Each level executes different mechanical, biological and chemical

functions. The whole bone which consists of 80% of cortical bone (~5-10% porosity) and 20% of cancellous bone (~50-90% porosity) is pictured at macro-scale level (Clarke, 2008; Bose et al., 2013). At micro-scale, hollow Haversian canal is surrounded by osteons comprises interstitial lamella in different direction. Lamella structure at sub micro-scale is formed by mineralized collagen fibrils. Hydroxyapatite crystal is embedded in mineralized collagen fibrils at nanostructure (Tadano and Giri, 2011).

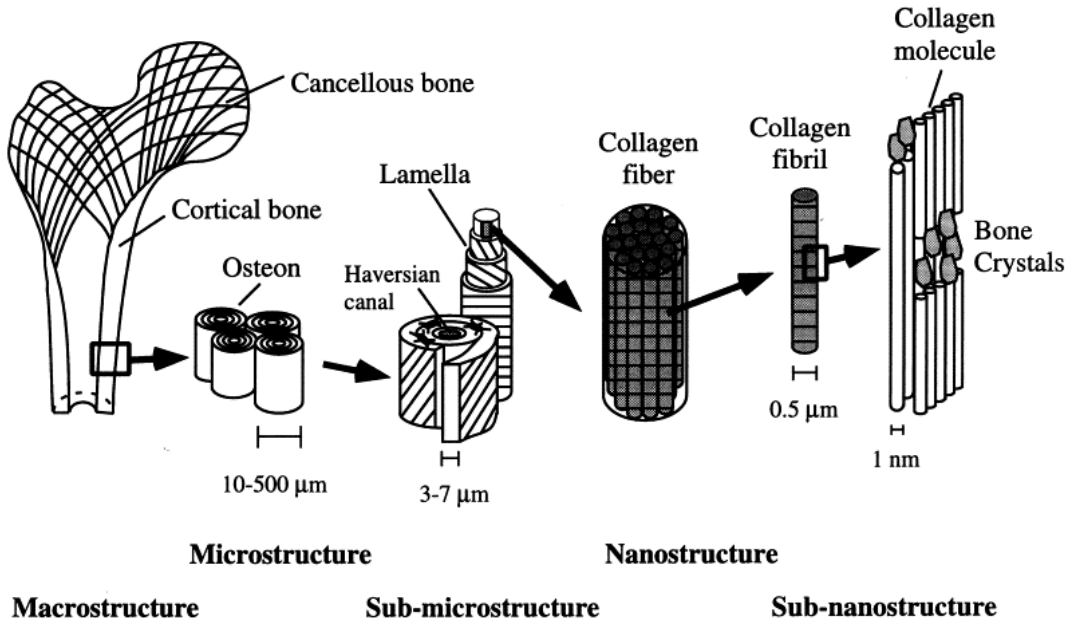

Figure 2.3 Hierarchical architecture of bone (Rho et al., 1998)

2.3.2 Bone Formation and Remodeling

Bone remodellng process is constantly carried out throughout lifetime to repair aged or microdamage in bones, maintain mineral homoeostasis and control bone marrow environment therefore human bones can stay healthy (Seeman, 2009). The bone is formed through enchondral and intramembranous processes. Enchondral process take place in long bones formation such as femur. This process involves mesenchymal cells secrete cartilage like matrix as precursor that in turn differentiate

into chondrocyte and gradually grow to become hypertrophic through chondrocyte proliferation. The osteobalasts is then absorbed to the matrix and lay down new bone (Little et al., 2011). Intramembranous process is to delevop bone (skull, calvicles and etc.) where mesenchymal cells is directly differentiate to become osteoblasts without cartilaginous phase (Akter and Ibanez, 2016). The process of bone remodeling is illustrated in Figure 2.4.

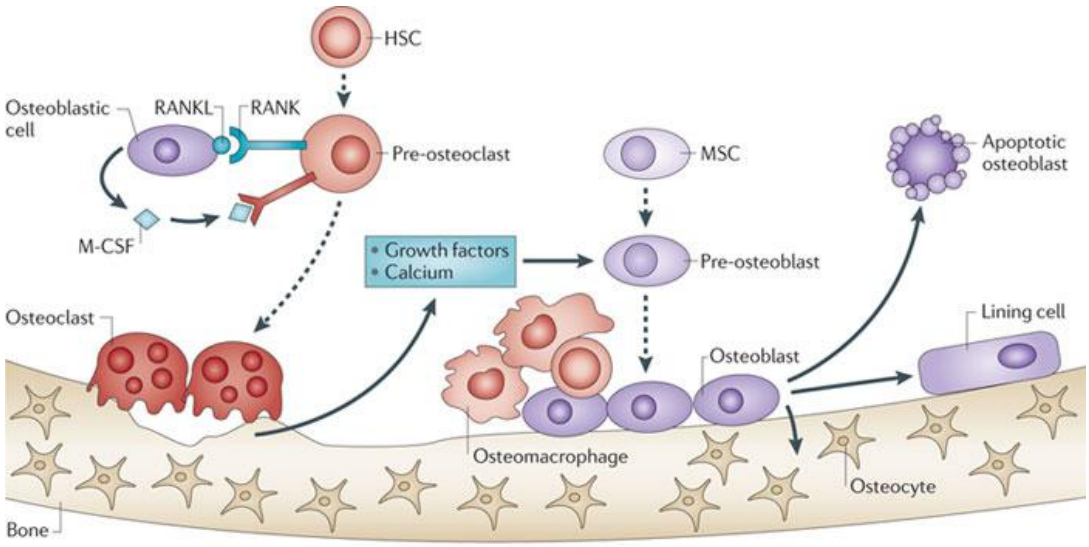

Figure 2.4 Process of bone remodel (Weilbaecher et al., 2011)

2.3.3 Mechanical Properties of Bone

The mechanical properties of bone are significantly varied based on age, sex, composition and structure of bone (Reznikov et al., 2014). Table 2.1 provides the summary of mechanical properties of human bone. Cortical both are much stronger and stiffer than cancellous bone due to its dense and compact structure. The cancellous bone has a spongy porous structure therefore the strength is dependent on the porosity and architectural arrangement (Wang et al., 2016).

Table 2.1 Mechanical properties of human bone (Bose et al., 2013; Wang et al., 2016)

	Porosity	**Modulus (GPa)**		**Strength (MPa)**	
Cortical bone	<10%	Longitudinal	17.9 ±3.9	Tension	135 ±15.6
				Compression	205 ±17.3
		Transverse	10.1 ±2.4	Tension	53 ±10.7
				Compression	131 ±20.7
Cancellous Bone	~50-90%	Vertebra	0.067 ±0.045		2.4 ±1.6
		Tibia	0.445 ±0.257		5.3 ±2.9
		Femur	0.441 ±0.271		6.8 ±4.8

2.4 Biomaterials

Biomaterial is a material used in various medical application for replacement of any diseased part or function of the body which interact with the living tissue to perform biological functions for the purpose of tissue regeneration (Wong et al., 2013). Some requirements must be considered during the designing of implants in the aspect of compatibility, manufacturing and mechanical properties (Migonney, 2014). The biomaterial must be harmless to human body so that the cell can adhere and function normally. Besides, it also must possess adequate mechanical properties depend on different medical application. In addition, it should have ease fabrication method for high reproducibility and consequently low production costs (Migonney, 2014; O'Brien, 2011).

Biomaterial has been categorized into three main classes which are metals, ceramics and polymers. Metallic biomaterials (stainless steel, titanium, cobalt-chromium alloys and etc.) are mainly used in load-bearing systems for instance hip and knee prostheses to fix bone fractures. Polymeric biomaterials such as ultra high molecular weight polyethylene, poly(methyl methacrylate), polyether ether ketone and etc. are used to replace joint components. Ceramic based biomaterials are

important for dental implants, hip and knee replacements (Mahyudin and Hermawan, 2016). Summary of three main classes of biomaterials is shown in Table 2.2.

Table 2.2 Summary of ceramic, polymeric, metallic and composite biomaterials

Type	Advantages	Disadvantages	Ref.
Ceramics	• High mechanical stiffness • Excellent biocompatibility • Good cell interaction	• Brittle • Difficult of shaping for implantation • Low elasticity • Not resilient	(O'Brien, 2011)
Polymer	• Resilient • Easy of fabricate • Lightweight	• Not strong • Time-dependant deformation	(Wong et al., 2013)
Metal	• Strong • Ductile	• Tend to corrode • Dense • Not bioactive	(Wong et al., 2013)

The challenge faced in bone tissue engineering is the capability to design materials that can bio-mimic natural bone in terms of chemical, mechanical and biological properties (Leonor et al., 2015; Olszta et al., 2007). The growing interest of using bioceramic and polymer as components to fabricate bone scaffold due to these material resembles chemical composition of real bone tissue matrix (Stevens, 2008).

2.4.1 Bioceramics

Bioceramics are defined as ceramics that are used to replaced, reconstruct or repair diseased part of body (Hench, 1998). Example of bioceramics includes calcium phosphate, alumina, zirconia and glass-ceramics. The following is some of the properties of bioceramics (Salinas et al., 2013; Chandramohan and Marimuthu, 2010):

- High hardness therefore good wear characteristics
- High Young's modulus, low elasticity and brittle
- Difficult to process due to very high melting points therefore high processing cost
- Excellent biocompatibility
- Bioinert e.g. Alumina, Zirconia
- Bioactive e.g. Hydroxyapatite and Bioglass

Bioceramics can be classified into three categories based on their reactivity which are biodegradable, bioactive and bio-inert as shown in Figure 2.5. Biodegradable bioceramics will degrade gradually and replace by newly formed bone (Poitout, 2016). Bioactive bioceramics is the ceramics materials that can react with physiological fluids and subsequently elicits the formation of tissues at its surface therefore providing the capability of direct bonding to living bone when implanted (Kutz, 2003; Ohtsuki et al., 2009). Bioinert ceramics is chemically very stable and has high mechanical strength (Poitout, 2016).

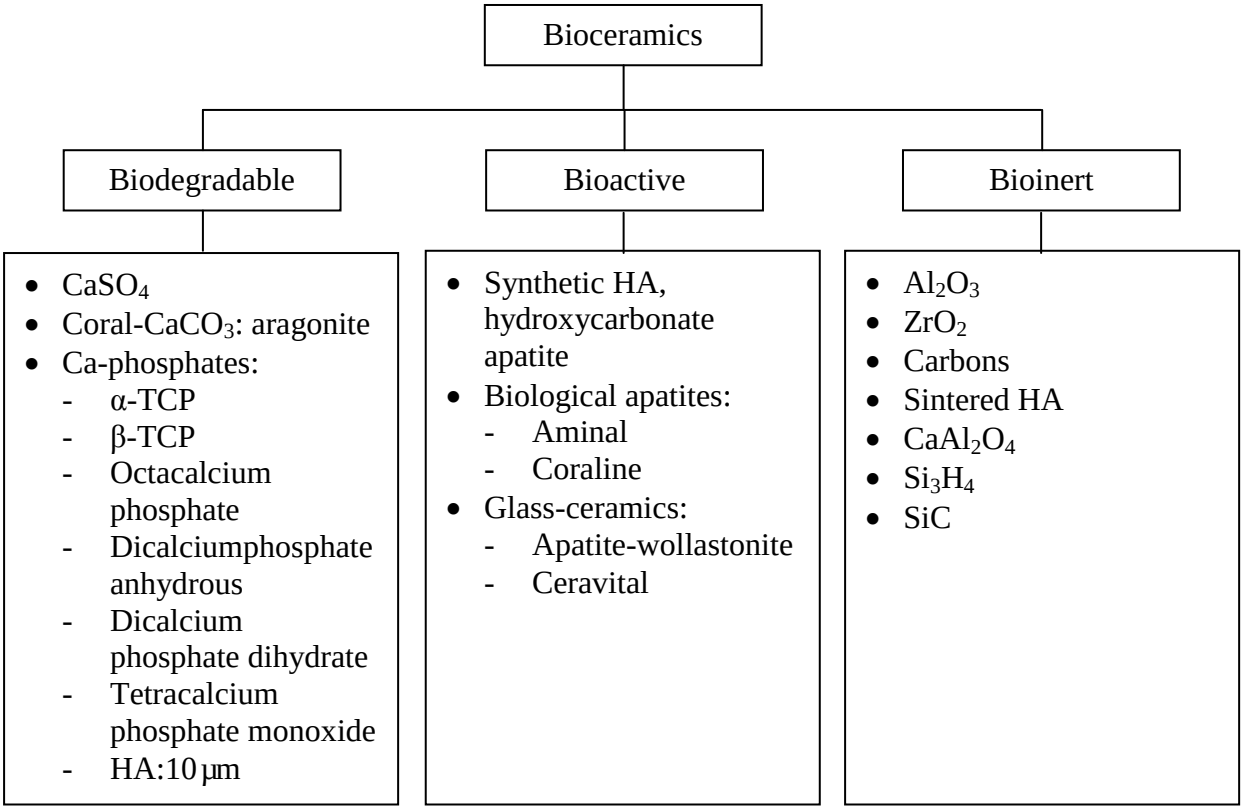

Figure 2.5 Classification of bioceramics (Salinas et al., 2013)

Among the bioceramics, calcium phosphate based ceramics gain high public noticeable and widely used in orthopaedic application due to their similar chemical composition to bone therefore exhibit excellent biocompatibility and bioactivity (Wang et al., 2012). Calcium phosphate ceramics also show osteoinductive characteristic as it can entrap and concentrate the growth factors that stimulate the differentiation of mesenchymal stem cells into osteoblasts (bone-forming cells) (Denry and Kuhn, 2016). Tricalcium phosphate and hydroxyapatite are alloplastic bone substitues that gain significant attention in bone tissue engineering application due to their biocompatibility and bioactivity characteristics (Grandi et al., 2011).

2.4.1.1 Hydroxyapatite

Hydroxyapatite as a calcium phosphate based bioceramic had been commonly used as a scaffold in tissue engineering. Hydroxyapatite is chemically similar to the mineralised phase of the bone in which the similarity accounts for its excellent osteoconductive and biocompatibility (Nandi et al., 2010; Monmaturapoj and Yatongchai, 2011).

Although this material exhibits excellent biocompatibility but the drawback is that it is too stable *in vivo* to be resorbed and substituted by natural host tissue (Yasuda et al., 2002). Despite having similar chemical composition as the bone, being low solubility has made it to less ideal for bone substitution (Vallet-Reg í and Gonz ález-Calbet, 2004).

2.4.1.2 Tricalcium Phosphate

Tricalcium phosphate, $Ca_3(PO_4)_2$ is a synthetic bone ceramic materials with Ca/P ratio of 1.5 which commonly used for bone replacement application (Habraken et al., 2016; Horowitz et al., 2009). Tricalcium phosphate shows higher solubility than hydroxyapatite and hence it can be resorbed after implantation (Sheth et al., 2017). Moreover, non-toxic biodegradation by-products and the release of calcium and phosphorus ions by resorption of TCP which aid in osteogenic activity for new bone formation make it more suitable used as material for bone reconstruction (Bose and Tarafder, 2012; Li et al., 2016).

Tricalcium phosphate (TCP) has three polymorphs which are β-, α- and α'-TCP. α- and β- forms of tricalcium phosphate are widely used while α'- forms is rarely used because it only exists at temperature above 1430 ℃ (Carrodeguas and De Aza, 2011). β-TCP is stable at ambient temperature whereas α-TCP is

thermodynamically stable from 1140 until 1470 ℃ (Cicek et al., 2011). Therefore, β-TCP is called as low temperature phase while α- and α'-TCP are high temperature forms of tricalcium phosphate (Carrodeguas and De Aza, 2011).

α- and β-TCP have similar chemical composition but they differ in other aspect such that structure, density and solubility. These characteristic will in turn affect their biological properties. β-TCP is commonly used in dental and othopeadic surgery as bone filler whereas α-TCP is used for component materials in calcium phosphate cements. α-TCP exhibits higher solubility than β-TCP which lead to higher biodegradation rate and therefore higher bioresorbability (Uchino et al., 2010). Among these three forms of triclalcium phopshate, β-TCP is more favourable to be used as a material for bone regeneration due to its chemical stability, mechanical strength and intermediate bioresoprtion rate (Miranda et al., 2006).

There are some researchers showed that β-TCP had been successfully used in posterolateral fusions, sinus augmentation and interference screws fixation (Thaler et al., 2013; Bettach et al., 2014; Wang et al., 2017). Kondo et al. (2006) and Ogose et al. (2006) reported that β-TCP showed osteoinductivity and bioresorption characteristics where β-TCP can be resorbed by the cell and followed by substitution of newly formed bone. Besides, Cai et al. (2009) investigated on the biological properties of β-TCP and it showed that β-TCP can support the MC3T3-E1 cells adhesion and proliferation.

2.5 Fabrication Methods of β-TCP Scaffolds

There are numerous methods to fabricate porous ceramic scaffold such as polymer sponge replication, direct foaming, sacrificial templating and gel casting (Jamaludin et al., 2015; Sopyan and Kaur, 2009). In polymer sponge replication

method, porous cellulosic substrates are impregnated with ceramic slurry (Sopyan and Kaur, 2009). Direct foaming produces porous ceramic structure by incorporating the air directly or air is generated in the ceramic suspension in which the structure of air bubbles is set to be maintained (Bhaskar et al., 2016). Sacrificial templating is a method where solid core template is dissolved or decomposed at high temperature which generates porous structure within the ceramic (Matsuda et al., 2016). Gel casting is another favourable method because it can produce dense and high mechanic strength scaffold, near net shape forming and easy to produce. Gel casting method is used to prepare porous ceramic structure by using gelling agents along with ceramic dispersed slurry which cause rapid setting of porous structure (Dash et al., 2015). In this study, gel casting was used to fabricate β-TCP scaffolds due to its ability to obtain high degree of geometry complexity, high homogeneity, good mechanical properties and capable for large scale production (Deng et al., 2017; Yuan et al., 2014).

Gel casting is a process which involves the dispersion of ceramic powder in aqueous monomer solution and subsequently the slurry was casted into a mould with desired shape. Afterwards, gelation occurs in the mould and followed by drying and sintering process (Gilissen et al., 2000). Higher density of ceramic scaffold can be obtained with the usage of higher solid loading. Therefore, the solid loading of ceramic can be controlled at favaourable amount. Higher solid loading can minimize the shrinkage of green body during drying and sintering process. In a result, cracking problem can be avoided (Ohji and Mritunjay, 2010).

By using gel casting method, β-TCP of various composition have been successfully fabricated into scaffold with porosity ranging from 86-88% with mean

pore size varied from 200-500 μm which considered adequate for bone tissue growth (Bose et al., 2012; Dorozhkin, 2010; Siqueira et al., 2017). The microstructure of β-TCP scaffold is shown in Figure 2.6. The pore structure and interconnectivity of β-TCP scaffolds were assessed by X-ray microtomography as shown in Figure 2.7.

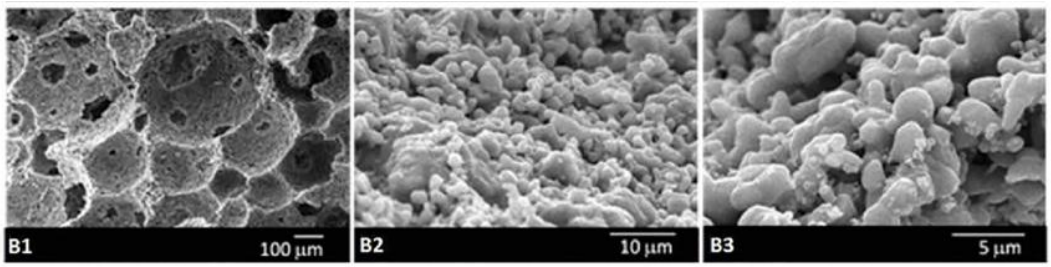

Figure 2.6 Scanning electron micrograph of porous β-TCP scaffold prepared by gel casting method (Siqueira et al., 2017)

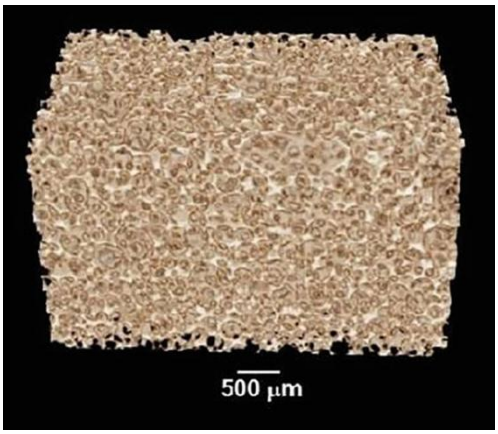

Figure 2.7 X-ray microtomography image of β-TCP scaffold prepared by gel casting method (Siqueira et al., 2017)

2.6 Biopolymer Coating Approach

Although calcium phosphate ceramics have good biological properties and have been proven successfully used in clinical applications, they are brittle and low strength consequently it can cause difficulty handling throughout implantation surgeon (Peroglio et al., 2007). Better mechanical properties and biological properties of ceramic scaffold can be attained by biopolymer coating of ceramic

scaffold. The reinforcing effect is achieved by the biopolymer coating which able to bridge the crack and consequently will undergo deformation and eventually broken. This make initiation and propagation of crack on the scaffold become harder (Mart ínez-V ázquez et al., 2014).

Scaffold can be coated with biopolymer using simple techniques which are dip coating, spray coating and spin coating (Smith and Lamprou, 2014). Favorable method to fabricate biopolymer coated scaffold can be done by immersing the scaffold in the biopolymer solution for adequate time to allow the biopolymer fully infiltrate the pores structure of scaffold. This dip-coating method (Figure 2.8) has gained high public attention due to its simplicity and versatility of the process (Philippart et al., 2014).

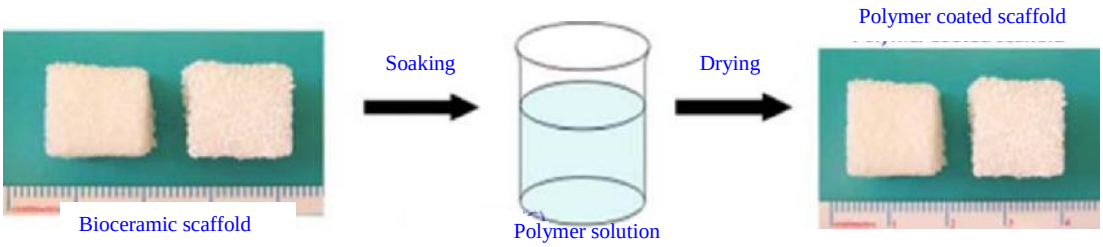

Figure 2.8 The dip-coating method (Mohamad Yunos et al., 2008)

Biodegradable polymer, also called as biopolymers are derived from natural sources which means that the polymers are able to decompose through enzymatic action of microorganisms with release carbon dioxide and water as the by-products (Csizmadia et al., 2013). Biodegradable polymer attracts public attention due to no toxic components are released suring its degradation process (Mendes et al., 2016). Biodegradable polymer can be categorized into two, which are natural sources based polymers and petroleum based synthetic polymers as shown in Figure 2.9. The natural resources based polymers from the chemical perspective include (Gupta and Kumar, 2007):

- Polysaccharides (starch, cellulose, lignin, chitin)
- Proteins (gelatin, casein, wheat, gluten, silk and wool)
- Lipids (plant oils including castor oil and animal fats)
- Polyesters produced by micro-organism or by plants (polyhydroxyalcanoates, poly-3-hydroxybutyrate)
- Polyesters synthesized from bio-derived monomers (polylactic acid)
- Miscellaneous polymers (natural rubbers, composites)

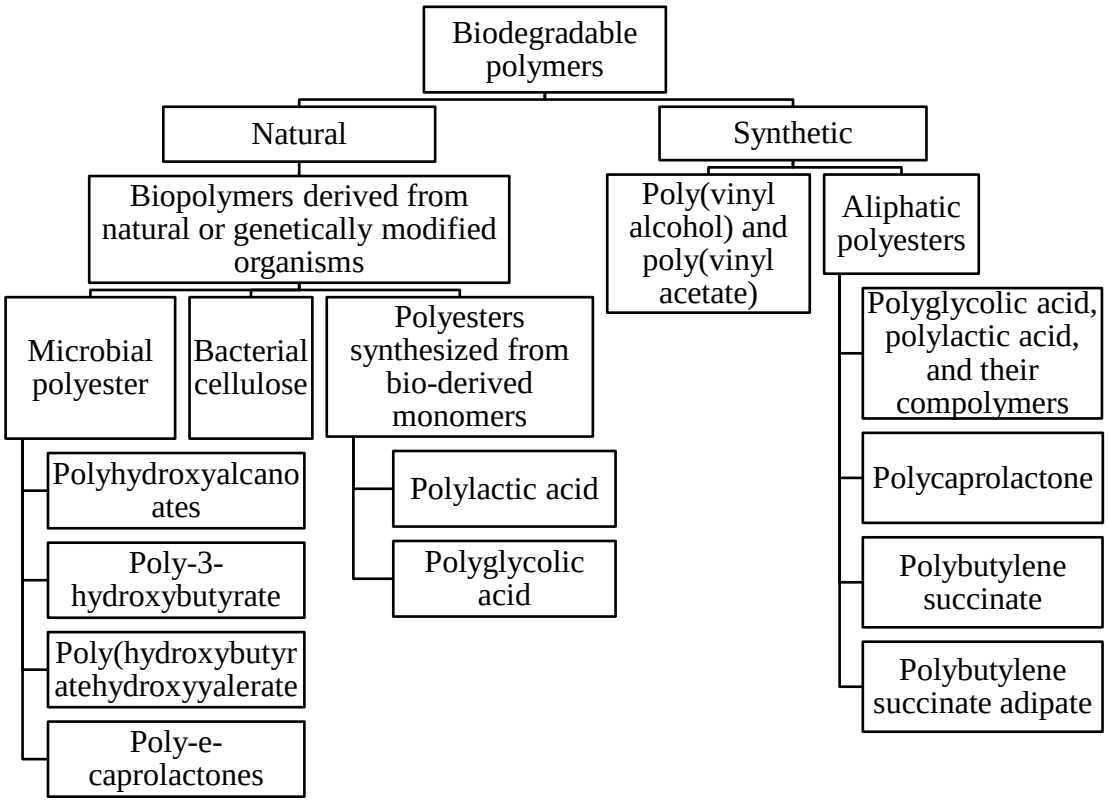

Figure 2.9 Biopolymer organization based on origin and production method (Mendes et al., 2016)