SCHOOL OF MATERIALS AND MINERAL RESOURCES ENGINEERING

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EFFECT OF MAGNESIUM ION (Mg^{2+}) SUBSTITUTION AND CALCINATION TO THE PROPERTIES OF BIPHASIC CALCIUM PHOSPHATE (BCP)

By

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DECLARATION

I hereby declare that I have conducted, compiled the research work and written the dissertation entitled **"Effect of Magnesium Ion (Mg^{2+}) Substitution and Calcination to the Properties of Biphasic Calcium Phosphate (BCP)"**. I also declare that it has not been previously submitted for the award of any degree or diploma or other similar title of this for any other examining body or University.

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LIST OF ABBREVIATIONS

β-TCP	Beta Tricalcium Phosphate
BCP	Biphasic Calcium Phosphate
CaP	Calcium Phosphate
DSC	Differential Scanning Calorimetry
FESEM	Field Emission Scanning Electron Microscope
FTIR	Fourier Transform Infrared Spectroscopy
HA	Hydroxyapatite
ICSD	International Centre of Standard Data
Mg-BCP	Magnesium-doped Biphasic Calcium Phosphate
TCP	Tricalcium Phosphate
TE	Tissue Engineering
TG	Thermogravimetry
XRD	X-ray Diffraction

LIST OF SYMBOLS

°	Degree
θ	Theta
%Vs	Percentage of shrinkage in volume
%T	Percentage of Transmittance
ρ_{bulk}	Bulk density

KESAN PENGGANTIAN ION MAGNESIUM (Mg^{2+}) DAN PENGKALSINAN KEPADA SIFAT-SIFAT KALSIUM FOSFAT DWIFASA (BCP)

ABSTRAK

Kalsium fosfat dwifasa didop magnesium (Mg-BCP) telah berjaya disintesis melalui kaedah mendakan berair pada suhu bilik. Objektif kajian ini adalah untuk mengkaji kesan penggantian ion Magnesium dan kesan pengkalsinan kepada struktur apatit. Serbuk BCP disintesis telah digunakan sebagai rujukan dalam kajian ini. Serbuk Mg-BCP disintesis telah dicirikan melalui analisis Permeteran Graviti Haba-Permeteran Kalori Pengimbasan Kebezaan (TG-DSC), Pembelauan Sinar-X (XRD), Kespektroskopan Jelmaan Fourier Infra-merah (FTIR) dan Mikroskop Imbasan Elektron Pancaran Medan (FESEM). Serbuk Mg-BCP disintesis telah difabrikasi kepada bentuk pelet berdiameter 13 mm melalui kaedah penekanan eka-paksi dan diikuti pengkalsinan pada tiga suhu berbeza (600 °C, 700 °C dan 800 °C) dalam atmosfera normal. Pelet Mg-BCP telah dicirikan melalui XRD (fasa), peratusan pengecutan isipadu, pengukuran ketumpatan pukal, ujian kekerasan Vickers dan FESEM (mikrostruktur). Keputusan XRD menunjukkan hanya fasa HA sahaja terhasil bagi pelet BCP. Walaubagaimanapun, bagi pelet Mg-BCP, campuran HA dan β-TCP telah berjaya terhasil bermula pada suhu 700 °C. Oleh itu, ia membuktikan bahawa penggantian Mg^{2+} ke dalam BCP mampu mengurangkan suhu transformasi fasa β-TCP. Tambahan pula, dengan pengahasilan β-TCP, nilai kekerasan pelet jatuh. Dalam antara pelet Mg-BCP, ketumpatan pukal dan nilai kekerasan didapati C6 Mg-BCP (dikalsin pada 600 °C) ialah 1.5534 g/cm^3 dan 43.8 HV masing-masing serta menunjukkan C6 Mg-BCP mempunyai sifat mekanikal yang lebih baik berbanding dengan C7 Mg-BCP (dikalsin pada 700 °C) dan C8 Mg-BCP (dikalsin pada 800 °C). Oleh yang demikian, penggantian Mg^{2+} dalam struktur apatit dan peningkatan suhu kalsin akan mengurangkan nilai kekerasan pelet Mg-BCP.

EFFECT OF MAGNESIUM ION (Mg^{2+}) SUBSTITUTION AND CALCINATION TO THE PROPERTIES OF BIPHASIC CALCIUM PHOSPHATE (BCP)

ABSTRACT

Magnesium-doped biphasic calcium phosphate (Mg-BCP) was successfully synthesized via aqueous precipitation method at room temperature. The objectives are to study the effect of substitution of magnesium ion and effect of calcination towards apatite structure. As-synthesized BCP powder was used as a reference in this research work. The as-synthesized Mg-BCP powder had been characterized via Thermogravimetry-Differential Scanning Calorimetry (TG-DSC), X-ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR) and Field Emission Scanning Electron Microscopy (FESEM). The as-synthesized Mg-BCP powder was then fabricated by uniaxial pressing method to form a 13 mm diameter pellet and followed by calcination at three different temperatures (600 °C, 700 °C and 800 °C) in normal atmosphere. The Mg-BCP pellets had been characterized by XRD (phase), volume shrinkage, bulk density measurement, Vickers hardness test and FESEM (microstructure). The XRD result shows that only HA phase was formed for BCP pellets. However, for Mg-BCP pellets, HA and β-TCP mixture was successfully formed started at 700 °C. Thus, it is proved that the substitution of Mg^{2+} into BCP able to lower the β-TCP phase transformation temperature. Furthermore, by the formation of β-TCP, the hardness value of pellets dropped. Among the Mg-BCP pellets, the bulk density and hardness values obtained for C6 Mg-BCP (calcine at 600 °C) were 1.5534 g/cm^3 and 43.8 HV, respectively and it showed that C6 Mg-BCP has better mechanical properties if compared to C7 Mg-BCP (calcine at 700 °C) and C8 Mg-BCP (calcine at 800 °C). Therefore, the substitution of Mg^{2+} in apatite structure and increasing calcination temperature will reduce the hardness value of Mg-BCP pellets.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Bone is a mineralized connective tissue that tough and rigid in skeleton system of human body. Bone applies an important role to supports the body weight, ensure skeletons have sufficient bearing capacity, assist movement activity, generates red and white blood cells and protection of internal organs (Wu, 2010; Florencio-Silva et al., 2015). There are 206 bones in a grown-up human body which can be ordered into two types, cortical bone and cancellous bone. Cortical bone otherwise called compact bone that has low porosity, while cancellous bone otherwise called spongy bone which has high porosity (Umadevi & Geethalakshmi, 2011).

Nowadays, accidents, injuries, diseases and obesity are a common phenomenon that lead to bone fracture. Fracture presences in bone are identified with the quality of bone which affected by a few biological factors, the mechanical behaviour and the microstructure (Kataruka et al., 2017). Along these lines, the comprehension of biological and mechanical properties of bone will help in growing better orthopaedic treatments.

Particularly, bone fractures were cured includes different medical surgical techniques, for a case, autografts, allografts and substitution implants (Bandyopadhyay et al., 2006). Bone grafting is a surgical method which involves replacement of missing bone with material from either patient's own body (autograft), from the donors (allograft) and from different species (xenograft) (Teresa Mao, 2013). Despite the fact that bone grafting had cured in numerous cases, this method is still faces with issues and disadvantages, as this method had limited lifespan, donor site morbidity, risks of disease transmission, may cause an inflammation and could not withstand mechanical forces

when being tested via in vivo testing (Kheirallah & Almeshaly, 2016). In this way, it is critical to deliver bone grafting materials that permitted fast cell development and re-establish the utilized of bone as functional load bearing that could withstand mechanical forces for ambulatory function (Bandyopadhyay et al., 2006).

In this way, recent advances from researchers in the development of Tissue Engineering (TE) has given the surgeon new options for surgeries. Bioactive materials (biomaterial) were introducing as new substituting materials that available to control action and response to the host tissue condition with a controlled chemical breakdown and resorption to eventually be supplanted by regenerating tissue (Kamath et al., 2014).

Biomaterials are generally described as any substance or combination of substances that can be utilized for any timeframe, which enhances or replaces partially or totally any tissue, organ or function of the body and furthermore with a specific end goal to maintain or improve the quality of life of the patients (Anusavice 2003). These days, biomaterial development for bone repair represents the most active research territory in the field of tissue engineering. One of the treatment to aid healing is to utilize synthetic biomaterials as the replacement or regenerate new tissue for the development of bone. This advancement was acquainted to overcome implants that have complicated issues, for example, costly, social implications and more troublesome procedure surgery (Mediaswanti et al., 2013). In this way, biomaterials used for implant must have some imperative properties, for example, biocompatibility, bioactivity, and resorbability which mimic with tissue and capability to help the body to recover itself post-implantation in order to long-term usage in the body without any rejection. (Patel & Gohil, 2012).

The most common classes of materials for biomaterials are metals, polymers, ceramics, and composite. Metallic biomaterials like titanium, tantalum, and magnesium

have been broadly utilized for biomedical applications, for example, load-bearing orthopedic applications and it demonstrated great biocompatibility. However, these materials often lack sufficient osseointegration capacity with regards to implant life span because of antagonistic reactions of some metallic ions with the surrounding tissues (Mediaswanti et al., 2013). As for this investigation, ceramic biomaterial is the main focus, where calcium phosphate (CaP) families were the most frequent utilized materials in biomedical applications (Teixeira et al., 2010).

Hydroxyapatite, HA ($Ca_{10}(PO_4)_6(OH)$) is a major inorganic constituent of bone. It has osteoconductive properties to bond directly to bone, both mechanically and chemically. Be that as it may, HA has an issue of poor biodegradation, which prevents the natural bone ingrowth for extended periods. Clinical application of bioactive hydroxyapatite can be enhanced with the bioresorbable tricalcium phosphate (TCP) for better bone regeneration (Cóta et al., 2016).

Tricalcium phosphate, TCP ($Ca_3(PO_4)_2$) is a bioactive and biodegradable material. TCP has four polymorphs: β, α, α' and γ phase. Each phase has different stable temperature. Among the four polymorphs of TCP, β-TCP is investigated intensively as a bioactive bone graft material because it needs low temperature to prepare and does not has an issue with high reactivity (Eliaz & Metoki, 2017)

HA and β-TCP are widely recognized as bioceramics for both dental and orthopedic applications due to their close chemical similarity with the inorganic component of vertebrate bone and tooth mineral (Sunarso, 2013). Currently, biphasic calcium phosphate ceramics (BCP) comprising a mixture of HA and β-TCP are considered better when compared with either single-phase HA or β-TCP components,

because of their unique dissolution characteristics in promoting new bone formation at the implant site (Sunarso, 2013).

Numerous properties of BCP, for example, bioactivity, biocompatibility, and solubility can likewise be enhanced by the consolidation of ions in their chemical composition (Kramer et al. 2014). Although the substitution does not intensely change the crystallographic properties of BCP, it is well known that small amounts of cations (Mg^{2+}, Sr^{2+}, Al^{3+} and K^{+}), or anions (F^{-}, SiO_4^{4-} and CO_3^{2-}) incorporated into the apatite structure play an important role in its biological performance. Cationic substitutions were done by substituting ions into calcium sites, while anionic substitutions were done by substituting ions into phosphate or hydroxyl sites for HA (Shepherd et al., 2012). Present of other follow elements such like magnesium for a case, give an effect on the performance of bone (Bandyopadhyay et al., 2006). Magnesium is a standout amongst the most studied ion and the fourth most abundant cation present in the human body. The substitution of Mg helps the mineralization of calcified tissue and specifically invigorates osteoblast proliferation, enhancing its mechanical properties (Brown et al., 2010).

1.2 Problem Statements

Diseased and damaged body parts, including bones, always have been a global problem. Thus, a demand for materials to improve the quality of life concerns the innovative use of specially designed biomaterials for the repair and reconstruction of diseased or damaged bones. Nowadays the focus has shifted towards bone replacement and repair materials, including bioceramics, that can mimic living tissues and assist in the healing process.

Bone commonly contains around 70% inorganic mineralized CaP phase and the remaining 30% for the most part involved natural non-mineralized collagen matrix (Cox

et al., 2014). CaP materials, including hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP), as well as biphasic calcium phosphate (BCP) composed of HA and β-TCP, are bioresorbable material and it is used for encourages new bone development by permitting the relocation, connection, and expansion of bone-framing cells. Therefore, CaP are the materials of choice to repair damaged bone (Wang et al., 2017).

For a long time, HA is an essential inorganic biomaterial which has pulled in the consideration of researchers related with biomaterials field because of its capability to stimulate ideal bone tissue recovery (Liu et al., 2001). However, researchers had demonstrated that HA varies marginally from normal bone apatite as for its chemical composition, percentage crystallinity and crystal structure (Thian et al., 2013).In direct contact biological framework, HA has a slow in vivo degradation and bioresorption rate to generate new growing bone tissue which restricts its applications for orthopaedic implants (Ebrahimi et al., 2012).

To overcome this issue, biphasic calcium phosphate (BCP) bioceramics are considered as a promising other option to HA-based bone substitute. BCP bioceramics belong to a group of bone substitute biomaterials that consist of an intimate mixture of HA and beta-tricalcium phosphate (β-TCP), $Ca_3(PO_4)_2$, of varying HA/β-TCP ratios (Sopyan & Rahim, 2012). In vivo studies have shown that BCP is considered better when compared with either single-phase HA or β-TCP components, because of their unique dissolution characteristics in promoting new bone formation at the implant site (Albayrak, 2016). HA has excellent biocompatibility and bioactivity and can be directly bonded to the host bone (Chen et al., 2017), β-TCP has suitable degradation rate that matches the growth rate of newly formed bone (Victoria & Gnanam, 2002), and by combination of the advantages of HA and β-TCP, BCP ceramics have been recently developed as an excellent starting material to prepare the bone tissue engineering. On the other hand, high

decomposition temperature needs for formation of β-TCP which is more than 800 °C (Tardei et al., 2006).

Hence, research into bone grafting materials, particularly, BCP, has been focused on the effects of ionic substitutions in order to control the phase transformation temperature of β-TCP. A wide range of different elements have been strategically incorporated into the structure of synthetic BCP. The incorporation or doping of ion into BCP alters the crystal structure and changes properties of the material.

The biological effects of magnesium (Mg) towards the roles in body functions have promoted the development of magnesium-based biomaterials for several decades. Until today, various modification of biomaterials has been performed through addition of biocompatible magnesium compound such as MgO, as well as through substitution of Mg^{2+} into the crystal structure of BCP. Mg^{2+} is additionally being brought into the BCP structure is important for several reasons including:

i. Increase of bioactivity
ii. Localized targeted delivery of the ions able to act on bone diseases such as osteoporosis
iii. Activating bone forming cells
iv. Controlling the phase transformation temperature

1.3 Research Objectives

There are three objectives that the project is concern. The objectives are:

a) To produce magnesium-doped biphasic calcium phosphate (Mg-BCP) powder and pellet.
b) To investigate the effect of Mg ion substitution on the as-synthesized and pellet magnesium-doped biphasic calcium phosphate (Mg-BCP).
c) To investigate the effect of calcination to the hardness value of magnesium-doped biphasic calcium phosphate (Mg-BCP) pellet.

1.4 Scope of Research

In general, this work can be divided into four main parts. Synthesis of biphasic calcium phosphate (BCP) and magnesium-doped biphasic calcium phosphate (Mg-BCP) powders were done in the first parts of this research work, next followed by characterization of as-synthesized powders. The third part of this work was about preparation of BCP and Mg-BCP pellets via uniaxial pressing method, which then calcined at 600 °C, 700 °C and 800 °C, and lastly followed by characterization of calcined BCP and Mg-BCP pellets. Figure 1.1 represent the flowchart for the scope of work involved in this research work.

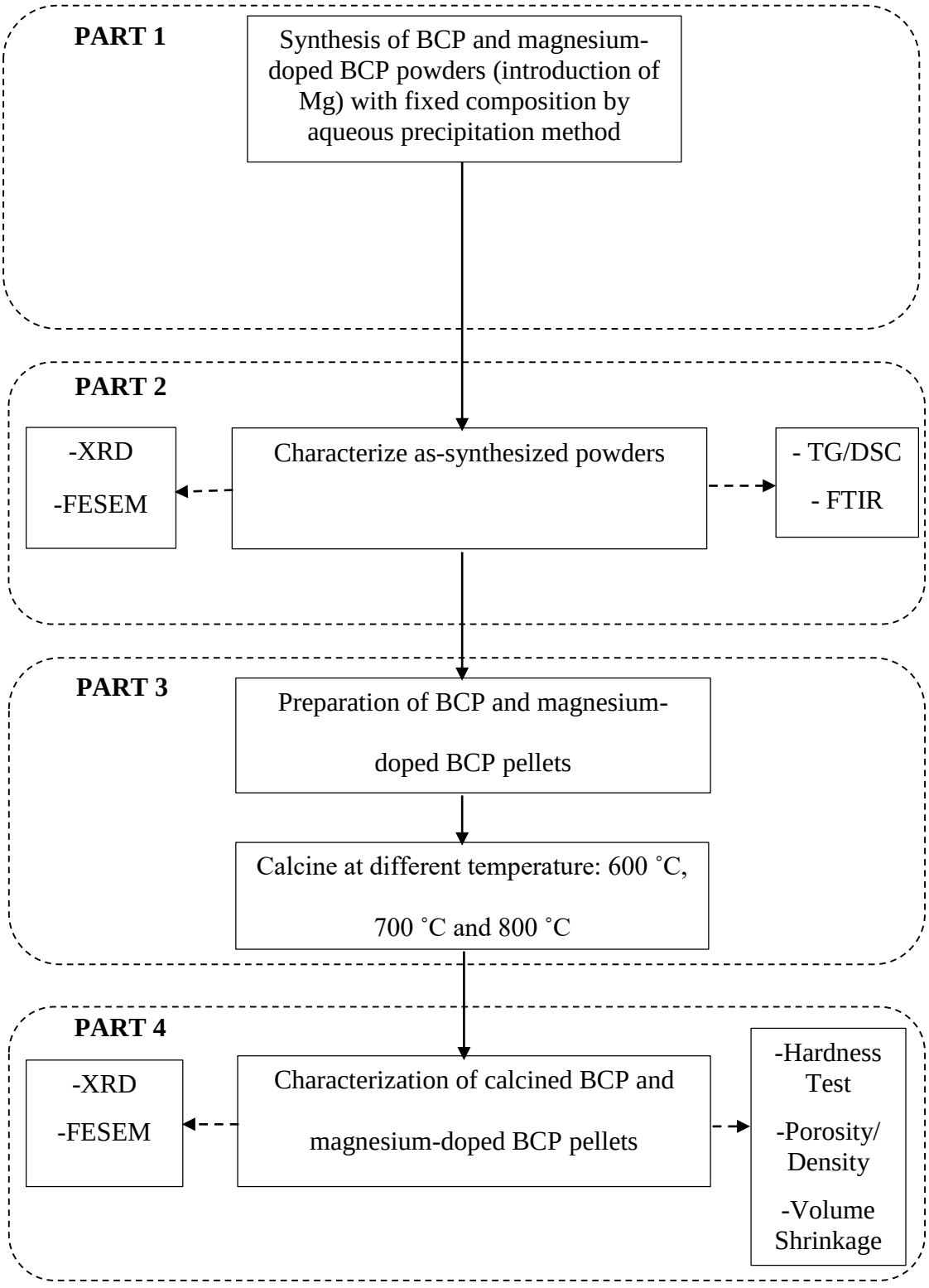

Figure 1.1: Flowchart of the research work

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Biomaterials began to be used in certain implant (Kumar et al., 2015) and since then, the development and applications of biomaterials have been continuously expended. Many definitions of the term biomaterials have been proposed. Park (2000) defined biomaterials as synthetic materials used to make devices to replace part of a living systems or to function in intimate contact with living tissue. Over the years, ceramic material had been developed as a biomaterial since it can replace lost tissue or organ structure (Adrezin, 2004).

The use of BCP has received large attention and being used as synthetic bone substitution material due to its chemical composition which is closely similar to that of the mineral constituent of natural bone and its excellent biocompatibility (van Esterik et al., 2016). However, unstable phase transformation happens in BCP. Thus, there have been initiative for researchers to investigate more about Mg^{2+} that have been introduce in BCP.

In general, this review starts with an overview of the function, structure and properties of natural human bone. Then, it followed by the development of bone graft as the main bone substitute material. The topic on bioceramic material, as a part of biomaterials, will be described in more detail starting with definition and examples. As BCP is the calcium phosphate based bioceramic, the properties of BCP bioceramic together with the explanation of the role of magnesium substitution had been presented thoroughly in this chapter. This is followed by a review into the types of processing methods to produce BCP and Mg-BCP.

2.2 Natural Human Bone

Bone may be simply described as a natural composite material which contain of organic and mineral phases. It is a complex mineralized living tissue that shows a certain degree of strength and rigid structure while maintaining some degree of elasticity (Kehoe & Eng, 2008). In human body, bone serves a number of functions (Umadevi & Geethalakshmi, 2011):

a) providing the cells in the marrow that differentiate into blood cells
b) acting as a calcium and phosphate reservoir
c) provide mechanical support and protect inner soft tissues likes heart and brain
d) act as weight bearing organ and responsible for almost all strength of human skeleton

2.2.1 Classes of Bone and Types of Bone Structure

There are five different classes of bones in human body such as long bones, short bones, flat bones, irregular bones and sesamoid bones. Long bones can normally have found on arms, legs, hands and feet. Next, short bones which found at the wrist and ankles while ribs, shoulder blades and hip bones are example of flat bones. Also, irregular bones found in the facial bones and sesamoid bones at special short bones and patella. Figure 2.1 shows different classes of bone according to shape (Umadevi & Geethalakshmi, 2011).

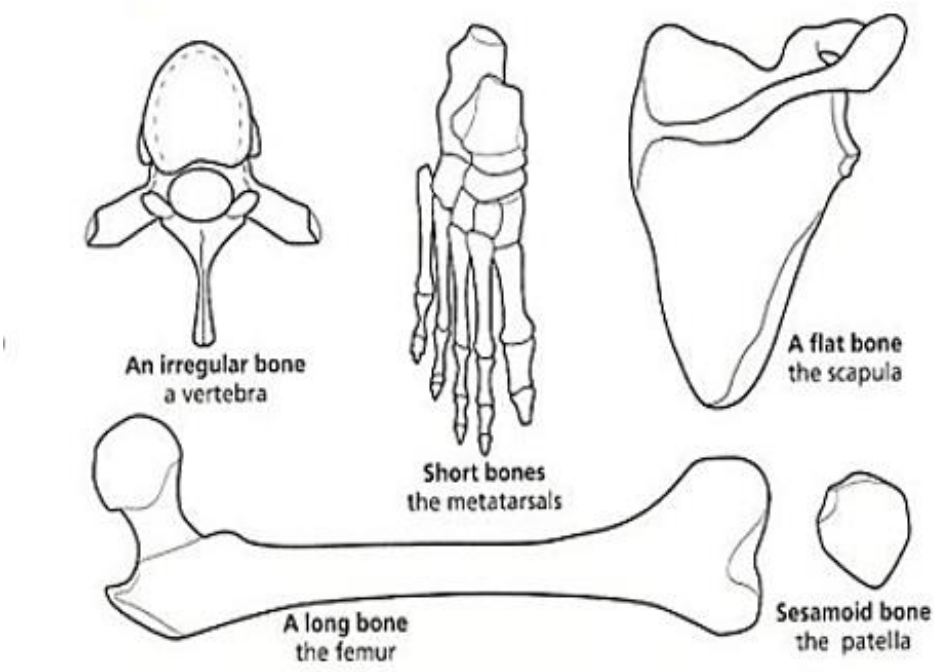

Figure 2.1: Bone classification according to shape (Umadevi & Geethalakshmi, 2011)

Generally, there are two types of bone structure as shown in Figure 2.2, which are cortical bone also known as compact bone and cancellous bone also known as spongy bone (Umadevi & Geethalakshmi, 2011). Both cortical bone and cancellous bone are form at the external shell and inner side of bone, respectively. Cortical bone is dense that consisting of parallel cylindrical units with porosity from 75% to 95%. Meanwhile, the cancellous bone is less dense that consisting of an array of rods and struts that form an open cell foam. The thickness of cortical bone is up to several tenths of a millimetre to several millimetres or even centimetres while around 55-300 μm thickness for cancellous bone (Eliaz & Metoki, 2017).

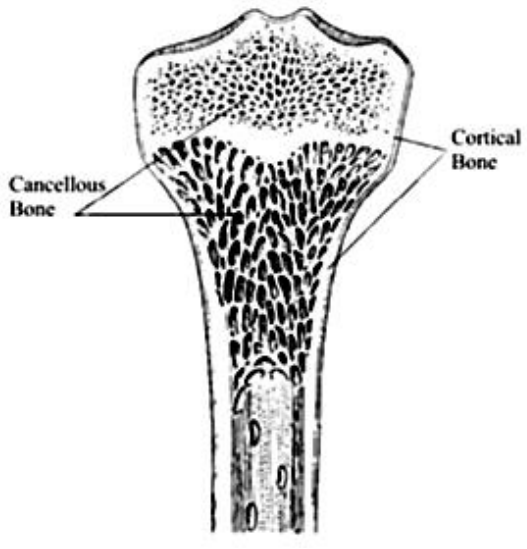

Figure 2.2: Natural bone structure (Umadevi & Geethalakshmi, 2011)

2.2.2 Bone Composition

Human bone composes of organic collagen fibres (organic phase), inorganic mineralized matrix (inorganic phase), and water. In bone, the percentage of inorganic mineralized matrix is approximately 60-65%, 20-25% made up of organic phase and remaining made up of water is shown in Figure 2.3 (Kehoe & Eng, 2008). Inorganic mineralized matrix of human bone consists of various bone minerals, where calcium phosphate as the main component while organic collagen fibers of human bone consists 90-95% of collagen especially collagen type I (Widyastuti, 2009). However, the quantitative composition of bone mineral is complex and can vary within one bone, between bones, between individuals, between species, with diet/with age and with pathological conditions (Boskey, 2013). In addition, the amount bone mineral constituent, proper arrangement and characteristics of everyone of bone mineral in view of quantity and quality will be define the properties of bone.

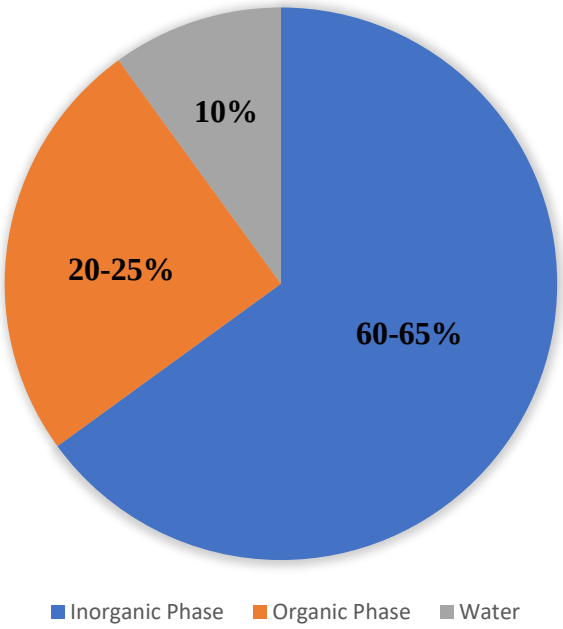

Figure 2.3: Phase present in human bone (Kehoe & Eng, 2008)

The main elements in bone mineral are calcium and phosphorus with 25.4 wt% and 11.6 wt% composition, respectively. There is also small number of other elements present in bone mineral composition. The composition of bone mineral is listed in Table 2.1 (Kehoe & Eng, 2008; Combes et al., 2016) .

Table 2.1: Composition of native bone (Kehoe & Eng, 2008; Combes et al., 2016)

Elements	Chemical Formula	Bone composition (wt%)
		Major Elements
Calcium	Ca	25.4
Phosphorus	P	11.6
Carbonates	CO_3^{2-}	5.6
Nitrogen	N	4.9
		Minor Elements
Magnesium	Mg	0.27
Sodium	Na	0.53
Chloride	Cl	0.13
Potassium	K	0.0047
Sulphide	S^{2-}	0.08
		Main Trace Elements
Cobalt	Co	$0 - 2.5\times10^{-6}$
Zinc	Zn	$0 – 3.9\times10^{-3}$
Strontium	Sr	0 – 0.05
Iron	Fe	7.6×10^{-3}
Fluoride	F^-	0.04
Aluminium	Al	2.9×10^{-3}
Lead	Pb	4.4×10^{-4}

2.3 Bone Graft

In any case, human skeletons can possibly helpless for bone damage or bone deformity caused by a wide range of ways, which trauma, infections, tumours and osteomyelitis (Han et al., 2017). Fracture happens in bone are related with the quality of bone which it impacted by mechanical behaviour, biological factor and the microstructure (Kataruka et al., 2017). Other than that, bone resorption is one of the bone imperfection which natural phenomenon occur because of aging (Teresa Mao & Kamakshi V., 2013).

Repairing bone defects includes different medical surgical treatments, for example bone graft substitution. A bone graft is a surgical system that generally used to settle issues with related to bones or joints because of trauma or problem joints (Teresa Mao & Kamakshi V., 2013). Bone graft substitution can be classified into three kinds, which are, autografts, allografts and xenograft are generally known for the successful treatments for bone substitution and regeneration (Whited et al., 2005). These three classes of bone graft substitution must follow four important characteristics required that listed in Table 2.2 (Hosokawa, 2013; Kheirallah & Almeshaly, 2016).

Table 2.2: Four important characteristics required for bone grafts (Hosokawa, 2013; Kheirallah & Almeshaly, 2016)

Characteristic	Explanation
Osteoconductive	Ability to support bone growth on a surgical part, during which pores, channels, and blood-vessels are formed within bone
Osteoinductive	Stimulation of osteoprogenitor cells to differentiate into osteoblasts then begin new bone formation.
Osteointegrative	Ability to direct contact of living bone to graft material
Osteogenesis	Provide formation of new bone by osteoblasts within the graft material

In fact, 3.5 million of bone graft procedures performed each year around the globe (Kheirallah & Almeshaly, 2016). Also, there are around 1 million bone-grafting surgery was done every year on the pelvis, spine and other extremities with estimation 700,000 joint substitution surgeries in United States and England (Shepherd et al., 2012).

2.3.1 Types of Bone Graft

2.3.1.1 Autogenous Bone Grafting

Autogenous bone grafting or also known as autograft is a method where the bone is transplanted taken from another part of the patient's own body. This method had been an effective treatment for bone defects for many years as it provide all three elements for

generating and maintaining bone tissue, which are osteogenetic progenitor cells, osteoinductive growth factors as well as oesteoconductive factors (Bellucci et al., 2011).

However, it has been pointed out that autogenous bone grafting has several drawbacks, including the patient has to endure two surgical interventions instead of one and there are general risks of infection such as HIV (Vallet-regí, 2010). Also, the volume of bone accessible to be used is limited and the collected bone is also limited in its form. Moreover, autograft is costly operation procedure, time consuming and sometimes can causes additional trauma (Wei et al., 2015).

2.3.1.2 Allogeneic Bone Grafting

Allogeneic bone grafting or also known as allograft is a method where the bone is transplanted taken from a donor's body from the same species. The differences between allograft and autograft is that the tissue source is not from the same individual (Rodríguez et al., 2015).

In practice, this method is rarely being used for bone defects due to rejection immune response and the risk of disease transmission (Kheirallah & Almeshaly, 2016). Mainly, transmission of tumour cells, bacterial and viral infection (hepatitis and HIV) can be carry by allogeneic bone grafting to the patients. Additionally, after transplantation, it also might cause infection to occur to the patients' body due to pathogen transmission from donor (Whited et al., 2005).

2.3.1.3 Xenogeneic Bone Grafting

Xenogeneic bone grafting or also known as xenograft is a method where the bone is transplanted taken from a donor's body from different species. Unlike allograft, xenograft has an advantage as it offers virtually unlimited source of organs (Rodríguez et al., 2015).

However, similar with allograft, xenograft also generally associated with potential infection that it may cause HIV and hepatitis transmission to the patients (Whited et al., 2005). Xenograft also less effective when compare with allograft regarding to high failure rate on antigenic response which related with the ability of a substance to trigger the immune response in a particular organism (Kheirallah & Almeshaly, 2016)

2.3.2 Important of Synthetic Bone

Bone graft techniques such as autograft, allograft and xenograft as mention in Section 2.3.1.1-2.3.1.3, respectively, have their own advantages and also disadvantages. In order to overcome the limitation of these techniques, development of synthetic bone shown positive results which it has potential alternative for supporting the newly formed bone tissue. Moreover, this synthetic bone has some advantages because of their unlimited availability, no risk of disease transmission and flexible in terms of composition without batch variability (Bellucci et al., 2011).

2.4 Biomaterials

As a simple definition, biomaterials can be defined as synthetic material used to replace part of a living system or to function as intimate contact with living tissues. The main purpose of biomaterials development is it can be used to be implanted in human body to replace the tissues defects and maintain or improve the life quality of the individual (Vallet-regí, 2010).

Based on Table 2.3, biomaterial can be categorized under four categories of reaction with tissue which are toxic material, bioactive material, bioinert material and bioresorbable material (Anusavice, 2003; Eliaz & Metoki, 2017). These categories listed are based on clinical requirement. In other word, the most important criteria which need to be considered are the materials used for implant should be: bioactive materials which

form direct chemical bonds with the bone or even with the soft tissue of a living organism; bioinert high strength materials, and various bioresorbable materials which are actively included in the metabolic processes of an organism with predictable results (Anusavice, 2003; Eliaz & Metoki, 2017). Basically, biomaterials are materials that will not cause negative response on the tissue after implantation and non-cytotoxic (Mediaswanti et al., 2013).

Table 2.3: Four categories of reaction between tissue and biomaterial (Anusavice, 2003; Eliaz & Metoki, 2017)

Classification	**Tissue Response**
Toxic material	Tissue dies
Bioinert material	Tissue form an adherent fibrous capsule around the implant
Bioactive material	Tissue for interfacial bond with implant
Bioresorbable material	Tissue eventually replace implant as new bone formation take place

Also, biomaterials can broadly be classified as biological biomaterials and synthetic biomaterials. Generally, synthetic biomaterials can be made up from four main classes of materials which are metals, polymers, ceramics and composites. Each classes of materials have some advantages and disadvantages in properties as well as processability and was exploited for different specific applications as shown in Table 2.4.

Table 2.4: Classes of materials used as biomaterials (Vallet-regí, 2010)

Materials	Advantages	Disadvantages	Examples
Polymers (nylon, silicone, rubber, polyester, etc)	Resilient, easy to Fabricate	Not strong, deforms with time, may degrade	Sutures, ear, nose, blood vessels, other soft tissues, hip socket
Metals (Ti and its alloys, Co-Cr alloys, Au, Ag, stainless steel, etc)	Strong, tough, Ductile	May corrode, dense, difficult to make	Joint replacements, dental root implants, bone plates and screws, pacer and suture wires
Ceramics (alumina, zirconia, calcium phosphates including hydroxyapatite, carbon)	Very biocompatible, inert, strong in Compression	Brittle, not resilient, weak in tension, difficult to make	Dental and orthopaedic implants
Composites (carbon-carbon, wire- or fiber-reinforced bone cement)	Strong, tailormade	Difficult to make	Joint implants, heart valves, bone cement, dental resin

2.5 Bioceramics

Ceramic whitewares is ceramic materials that used to produce tableware, sanitary ware and tiles. Specialized or advanced ceramic otherwise called fine ceramics is ceramic materials used to produce semiconductors, structural ceramics and bioceramics which utilized for biomedical application (Best et al., 2008).

By and large, bioceramics are alluded to biocompatible ceramic materials that constantly pertinent for biomedical or dental applications (Nasseh, 2009). In another word, ceramic biomaterials are utilized for repair and replacement of diseased and damaged parts of musculoskeletal systems (Adrezin, 2004). Additionally, ceramics are broadly utilized contrasted with metals because of biological inertness of ceramic as biomaterials for medicine and dentistry for as long as three decades (Thamaraiselvi & Rajeswari, 2004). These days, bioceramics are broadly utilized for medical applications, for example, to a great extent for implants in orthopedics, maxillofacial surgery and for dental implants.

There are three frequently classification that are related to bioceramics and this classification is according to the biological reactivity in the body. First classification is biological inert high strength ceramics. Alumina (Al_2O_3) and zirconia (ZrO_2) are the example of materials that related to bioinert characteristic. The most common response of tissue to an implant from these materials is the formation of non-adherent fibrous capsule. The tissue attempts to reject the implant by creating a barrier around it (Chevalier & Gremillard, 2009). Second, surface bioactive ceramics also one of bioceramics types. Example of materials are silica (SiO_2), calcium oxide (CaO) and sodium oxide (Na_2O). This material being implant and form direct chemical bonds across the interface between the bone or even soft tissue of living organism with implant to prevent motion between the two surfaces (Thamaraiselvi & Rajeswari, 2004). Lastly, bioresorbable ceramics which referred to the implant material that able to be dissolved by the surrounding body tissue with predetermined rate or by controllable manner (Popov et al., 2014). As example, crystalline HA and BCP are related to bioresorbable characteristic (Best et al., 2008; Popov et al., 2014).

2.6 Calcium Phosphate Bioceramics

2.6.1 Calcium Phosphate

Calcium phosphate refers to a family of inorganic compound minerals that have a main constituent of calcium ion (Ca^{2+}) and phosphate ion (PO_4^{3-}), the main inorganic phase of bone. Calcium phosphate ceramics are categorized into certain types including tricalcium phosphate ($Ca_3(PO_4)_2$), dicalcium phosphate anhydrous ($Ca_2H_2(PO_4)_2$) and other apatite group (Eliaz & Metoki, 2017).

Generally, calcium phosphate minerals were categorized according to their calcium over phosphorous (Ca/P) ratio which usually in between 0.5 to 2.0. Furthermore, each category of calcium phosphate has significant different in properties as a function of composition and phase. Table 2.5 shows the different types of calcium phosphate minerals with their properties (Dorozhkin & Epple, 2002; Boanini et al., 2010).

Table 2.5: Properties of biologically relevant calcium phosphates (Dorozhkin & Epple, 2002; Boanini et al., 2010)

Compound name	Abbreviation	Formula	Ca/P ratio	pH stability	Crystal structure	Density
Monocalcium phosphate monohydrate	MCPM	$Ca(H_2PO_4)_2.H_2O$	0.5	0.0 - 2.0	Triclinic	2.23
Monocalcium phosphate anhydrate	MCPA	$Ca(H_2PO_4)_2$	0.5	[a]	Triclinic	2.58
Dicalcium phosphate dehydrate (brushite)	DCPD	$CaHPO_4.2H_2O$	1.0	2.0 - 6.0	Monoclinic	2.32
Dicalcium phosphate anhydrate (monetite)	DCPA	$CaHPO_4$	1.0	[a]	Triclinic	2.89
Octacalcium phosphate	OCP	$Ca_8(HPO_4)_2(PO_4)_4.5H_2O$	1.33	5.5 - 7.0	Triclinic	2.61
α-tricalcium phosphate	α-TCP	$\alpha\text{-}Ca_3(PO_4)_2$	1.5	[b]	Monoclinic	2.86
β-tricalcium phosphate	β-TCP	$\beta\text{-}Ca_3(PO_4)_2$	1.5	[b]	Rhombohedral	3.07
Amorphous calcium phosphate	ACP	$Ca_x(PO_4)_y.nH_2O$	1.2 – 2.2	[e]	Hexagonal	1.75
Calcium-deficient hydroxyapatite	CDHA	$Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$ $(0<x<1)$	1.5 - 1.67	6.5 - 9.5	Hexagonal	1.59
Hydroxyapatite	HA	$Ca_{10}(PO_4)_6(OH)_2$	1.67	9.5 - 12	Monoclinic or hexagonal	3.16
Tetracalcium phosphate	TTCP	$Ca_4(PO_4)_2O$	2.0	[b]	Monoclinic	3.05

[a] Stable at temperature above 100 °C. [b] Cannot be precipitated. [c] Always metastable

2.6.2 Hydroxyapatite (HA)

Hydroxyapatite (HA) is one of member in apatite group ceramics which is the main constituent of inorganic part of the bone structure with a stoichiometric Ca/P ratio of 1.67 (Sibte et al., 2013). HA has chemical formula $Ca_{10}(PO_4)_6(OH)_2$ (Gonzalez Ocampo et al., 2016).

Over the years, HA has been recognized as an excellent biomaterial since HA has widely used in bone reconstructive surgery due to outstanding biological compatible with living cells hence able to permit new bone formation when implanted in a bone defect site (Thian et al., 2013). HA is well known for its good stability, bioactivity, biocompatibility, non-toxicity and osteoconductivity properties (Baba Ismail et al., 2017) and directly associated to the mineralization process in biological systems (Moreno et al., 1968). Component of mineral structure in artificial HA seems to have similarity with living body (bone and teeth) as presented in Figure 2.4 (Sopyan et al., 2007).

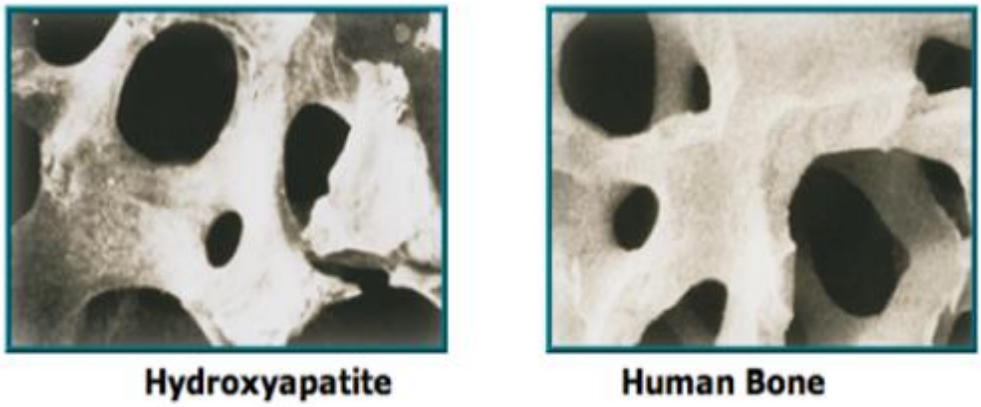

Figure 2.4: Comparison between hydroxyapatite and human bone (Sopyan et al., 2007).

HA is greatly being studied by researchers over the past few decades as a biomedical material. As an outcome of huge favourable to be capable stimulate development of immature cell and biologically active inside living cells, HA are widely being utilized as the high-quality biomaterial either in orthopaedics and dental