SYNTHESIS AND CHARACTERIZATION OF Mg-SUBSTITUTED BIPHASIC CALCIUM PHOSPHATE

NUR ATIQQAH BINTI MOHD. MOSLIM

UNIVERSITI SAINS MALAYSIA

2019

SYNTHESIS AND CHARACTERIZATION OF Mg-SUBSTITUTED BIPHASIC CALCIUM PHOSPHATE

by

NUR ATIQQAH BINTI MOHD. MOSLIM

Thesis submitted in fulfilment of the
requirements for the degree of
Master of Science

August 2019

ACKNOWLEDGEMENT

First of all, I would like to thank god Allah S.W.T. for his blessing so this thesis could be finished as I planned in the requirement to complete my Master's Degree. I would like to express my thanks and gratitude to my supervisor, Dr. Shah Rizal Kasim as well as my co-supervisor, Dr. Nurazreena Binti Ahmad for the support and guidance. I would also like to thanks the Dean of School of Materials and Mineral Resources Engineering, USM, Professor Dr. Ir. Zuhailawati Binti Hussain for providing opportunity and facilities for me to complete my research studies here. I am feeling oblige in taking the opportunity to sincerely thanks all the staff of School of Materials and Mineral Resources Engineering and Universiti Sains Malaysia wholly for any of their participation in completing my research work and dissertation. A big thanks to Ministry of Education for the Fundamental Research Grant (FRGS) 203.PBAHAN.6071354 which providing the financial support for this research. I would also like to thanks all of my friends and also individuals who have contributed to my work directly or indirectly. Thank you for your good deeds and enlightenment all the time. I pray that God would ease your journey. Last but not least, I would like to express my special thanks of gratitude towards my parents for their endless support in every aspect. I am greatly indebted for their love and encouragement up to this stage. Thank you so much!

TABLE OF CONTENTS

Appendix F: Information Required to Generate the Crystal Structure of the Calcined L-MgBCP1.60-6C Sample (β-TCP)

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

°C	Degree Celsius
mol %	Moles percent
nm	Nanometer
wt %	Weight percent
ml	millimeter
°C/min	Degree Celsius per minute
θ	Theta
λ	Lambda
Å	Angstrom
°/min	Degree per minute
°	Degree
cm-1	Reciprocal wavelength
g	gram

LIST OF ABBREVIATIONS

α-TCP	Alpha-tricalcium phosphate
β-TCP	Beta-tricalcium phosphate
BCP	Biphasic calcium phosphate
Ca	Calcium
CaP	Calcium phosphate
Ca/P	Calcium to phosphorus ratio
CDHA	Calcium deficient hydroxyapatite
CPC	Calcium phosphate cement
EDX	Energy dispersive X-ray
FTIR	Fourier transform infrared
HA	Hydroxyapatite
ICDD	International Centre of Diffraction Data
ICP	Inductively coupled plasma
ICP - AES	Inductively coupled plasma – atomic emission spectroscopy
M	Ca + Mg
M/P	(Ca + Mg) to P ratio
Mg	Magnesium
P	Phosphorus
SEM	Scanning electron microscopy
TCP	Tricalcium phosphate
TEM	Transmission electron microscopy
TGA	Thermogravimetric analysis
XRD	X-ray diffraction

SINTESIS DAN PENCIRIAN PENGGANTIAN Mg DALAM KALSIUM FOSFAT DWIFASA

ABSTRAK

Sampel kalsium fosfat dwifasa (BCP) tanpa dan terdop magnesium (Mg) disintesis melalui kaedah pemendakan pada suhu bilik. Penghasilan BCP yang terdop Mg dipertimbangkan kerana Mg merupakan antara unsur utama yang terdapat dalam tulang manusia. Oleh itu, kajian ini memberi penekanan terhadap kesan penggantian Mg kepada pembentukan fasa dengan mengambil kira tiga jenis pemboleh ubah, iaitu kepekatan ion Mg, nisbah molar antara bahan prapenanda, dan suhu kalsin. BCP tanpa dan terdop Mg yang telah disintesis dan dikalsin kemudiannya melalui proses perincian. BCP tanpa Mg yang disintesis menunjukkan pembentukan fasa monetit dan hidroksiapatit (HA) manakala BCP terdop Mg mengandungi fasa HA sahaja. BCP tanpa dan terdop Mg yang dikalsin pada suhu 600 °C menunjukkan pembentukan dwifasa HA dan beta-trikalsium fosfat (β-TCP). BCP dengan kandungan ion Mg tertinggi menghasilkan fasa β-TCP terbanyak (75.3 % berat) manakala sampel BCP tanpa Mg mempunyai fasa β-TCP yang terendah (20.5 % berat). Hal ini menunjukkan peningkatan pembentukan fasa β-TCP adalah seiring dengan peningkatan kandungan ion Mg. Perbezaan nisbah molar antara bahan prapenanda tidak memberi kesan yang signifikan terhadap pembentukan fasa berbanding dengan kesan kandungan Mg. BCP yang dikalsin pada suhu 500 °C menunjukan pembentukan fasa β-TCP yang lebih rendah berbanding BCP yang dikalsin pada suhu 600 °C. Dapatan ini menunjukkan bahawa peralihan fasa BCP dari HA ke β-TCP telah dipertingkatkan apabila didedahkan pada suhu kalsin yang tinggi.

SYNTHESIS AND CHARACTERIZATION OF Mg-SUBSTITUTED BIPHASIC CALCIUM PHOSPHATE

ABSTRACT

Sample without magnesium (Mg) and Mg-substituted biphasic calcium phosphate (BCP) were synthesized through precipitation method at room temperature. The substitution of Mg in BCP were considered due to Mg being one of the main element found in human bones. Hence, this study emphasize more on the effect of Mg substitutions towards phase formations with regards to three parameters which are the concentration of Mg ions, the molar ratio between precursors, and the calcination temperature. The as-synthesized sample without Mg BCP shows the formation of monetite and hydroxyapatite (HA) phase while the Mg-substituted BCP show only HA phase exist. As for the calcined BCP powders at 600 °C, both without Mg and Mg-substituted samples shows biphasic formation of HA and beta-tricalcium phosphate (β-TCP). BCP with the highest Mg ions concentration exhibit the highest β-TCP phase formation (75.3 wt %) while the without substituted BCP sample has the lowest β-TCP formation (20.5 wt %). It shows an increasing trend of β-TCP phase with the increase of Mg concentration. The different molar ratio between precursors does not give subtle effect on the phase formations as compared to the effect of Mg concentrations. BCP powders calcined at lower temperature of 500 °C shows less formation of β-TCP phase when compared to sample calcined at 600 °C. This results proved that the phase transition of BCP from HA to β-TCP were enhanced when subjected to high calcination temperature.

CHAPTER ONE

INTRODUCTION

1.1 Research background

It is widely known that the main purpose of the skeleton is to provide support for our body. It also helps to protect our internal organs and fragile body tissues. Apart from containing red bone marrow that produces blood cells, bones provide a structure for muscles attachment which could enable movement of the body. It contains their own nerves and blood vessels, and they act as a reservoir to stores body minerals like calcium and others. Bones are made of a mixture of substances such as collagens that gives them strength and tons of living cells which help them to grow and repair themselves in the case of fracture from accident or diseases. It can be fed and kept healthy through exercising and consuming nutritious food rich in calcium and vitamin D or else it may suffer from diseases such as osteoporosis (OrthoInfo, 2014).

While they are relatively strong, there is still a limit for bones to experience forces or trauma before breaking or fails. It is very important for fractured bones to heal properly in its original condition. Some injured bones can be healed by simply wearing a cast, or in more serious cases, it requires surgery considering the use of bone substitutes. There are four common bone substitutes known as alloplast (synthetic bone substitute), xenograft (harvested from bovine bone or coral), allograft (harvested from one individual and implanted into patient of the same species), and autograft (bone tissue transferred from different part of the same individual) (Hench, 2013). Specifically, alloplast enables us to tailor its structure and material properties as desired by us (Ducheyne, 2011). This type of grafts may be made from hydroxyapatite (HA),

bioactive glass, tricalcium phosphate (TCP), polymethylmethacrylate (PMMA) or polyhydroxyethylmethacrylate (PHEMA).

It is acknowledged that all of the aforementioned bioceramics contains traces of calcium (Ca) and phosphorus (P); as in our natural bones (Stipniece, 2016). It should be noted that most of the alloplast bone graft being used are bioceramics like HA and TCP, due to its osteoconduction, hardness and acceptability by bone. HA exhibit close resemblance to chemical composition of natural bone; high biocompatibility characteristic that is needed in bone regeneration while beta-tricalcium phosphate (β-TCP) provide the high solubility required for bone growth. However, the low resorption of HA into the body could hinder new bones formation and become one of its limitations for rapid healing while the bioresorbability of β-TCP is too fast and not desired too as it will cause the bone graft to collapsed (Kanchana & Sekar, 2012).

To overcome these complications, biphasic calcium phosphate (BCP) have been implemented in bone replacement. Since BCP contain a mixture of both HA and β-TCP, the properties of BCP should concerning both phases, depending on their compositions. The presence of both phases in BCP compliment both of their advantages and disadvantages as discussed previously. Through various studies regarding BCP, this bioceramic has been synthesized in the form of powder (Zyman *et al.*, 2008; Kanchana & Sekar, 2012; Kim *et al.*, 2012; Prezas *et al.*, 2017). Some of the methods used widely to synthesize BCP powder are sol-gel method, precipitation, hydrothermal or through mechanical mixing (Kim *et al.*, 2012). Among all, chemical methods receive more attention. During synthesizing of BCP powder using wet chemical method such as sol-gel and precipitation, it is very crucial to control the ratio between two dominant ion precursor; which is calcium ion (Ca^{2+}) and phosphate ion ($(PO_4)^{3-}$) (Kim *et al.*, 2017). To obtain BCP, the molar ratio of calcium to phosphate (Ca/P) is normally fixed in

between 1.5 to 1.67 (Stipniece, 2016). Ca/P molar ratio closer to 1.5 will produce BCP with a high amount of β-TCP while Ca/P molar ratio closer to 1.67 will produce BCP with a high amount of HA in it. This is due to the synthesizing molar ratio of pure β-TCP and pure HA are 1.5 and 1.67 respectively (Bouler *et al.*, 2017).

It should be noted that BCP bioceramics contain absolutely only Ca and P elements while in natural bones, there exist various other traces of elements such as magnesium (Mg), iron (Fe), zinc (Zn), sodium (Na) and potassium (K) (Gomes *et al.*, 2017; Gomes *et al.*, 2012; Kannan *et al.*, 2008). Hence, substitution of a little amount of element is considered in recent studies and becomes a trend (Gomes *et al.*, 2017; Kanchana & Sekar, 2012; Kannan *et al.*, 2010) . Mg is one of the most abundant element present in the human body as in enamel (0.44 wt. %), dentin (1.23 wt. %), and bone (0.72 wt. %) (Webler *et al.*, 2015). Mg ions (Mg^{2+}) are known to affect the metabolism of bone considering its osteoblast and osteoclast properties. The deficiency of this ion in bone would result in diseases such as osteoporosis (Geng *et al.*, 2016). Based on a cell culture study, the Mg-substituted BCP bio eramic has no effects on cytotoxicity, thus promising that this material can be used in biomedical applications (Webler *et al.*, 2015).

Some studies regarding Mg substitutions show that this element helps in destabilizing phase composition of the BCP bioceramic when subjected to heat treatments (Geng *et al.*, 2016; Kanchana & Sekar, 2012; Kim *et al.*, 2013; Stipniece, 2016; Webler *et al.*, 2015). BCP were normally subjected to heat treatment such as calcinations for the purpose of phase changes and sintering for densifications. Most of the calcination process were done at low temperature of 500 – 1000 °C, while sintering process were done above 1000 °C. It is known that the decomposition of HA to β-TCP phase in BCP occur when it is being heat treated up to 850 °C (Kokubo, 2008).

1.2 Problem statement

Precipitation followed by calcinations is the common synthesis route to produce BCP powder and it is highly dependent on the starting Ca/P ratios (Ebrahimi *et al.*, 2017). The advantages of this method include simple equipment and ability to produce uniform fine particles (Ebrahimi *et al.*, 2017). The calculated Ca/P ratio before synthesizing normally would differ with the calcined BCP bioceramics (Marchi *et al.*, 2009; Webler *et al.*, 2015), therefore it is important to control this ratio because it will determine the properties of BCP bioceramic produced. Apart from the Ca/P ratio consideration, the presence of substituted elements also would significantly affect the properties of BCP produced. The presence of other elements in our bones such as Mg, Fe, Zn and others need to be considered. For example, Mg serves the importance as one of the abundant element in human body and thus affecting bone metabolism (Webler *et al.*, 2015). The substitution of Mg to the apatite has attracted researchers due to its significant impact on mineralization (formation of β-TCP and HA phase) process and also its influence in crystal formation and growth (Araújo *et al.*, 2009; Stipniece, 2016). The effects of substitution of Mg^{2+} ions on phase formations still need to be investigated in order to grasp a better understanding on the substitution of Mg in BCP.

However high temperature calcination (above 600 deg C) to decompose HA to BTCP of HA in BCP bioceramic was commonly being studied occur when it is being subjected to calcination process (Prezas *et al.*, 2017; Sunarso *et al.*, 2013; Webler *et al.*, 2015). Nevertheless, there are still studies being done on the phase changes in BCP sample calcined at low temperature of 600 °C (Gozalian *et al.*, 2011; Gozalian *et al.*, 2012; Sopyan and Rahim, 2012), the phase changes were rarely being studied and discussed (Gomes *et al.*, 2018). It is known that the β-TCP phase is not stable up to 850

°C and undergo several phase changes. A study reported that the non-stoichiometric calcium-deficient hydroxyapatite (CDHA) would transform to β-TCP phase in the range of 450 – 500 °C (Victoria & Gnanam, 2002).

Mg^{2+} ions has known to preferably being substituted into β-TCP phase rather than HA phase in BCP (Kannan *et al.*, 2005). However, the ability of Mg^{2+} ions to reside in BCP was not discovered extensively and thus requiring structural investigation. It should be acknowledged that there are certain limit amount of Mg that could be substituted in BCP which relates to substitution mechanism of Mg (Araújo *et al.*, 2009). The study on crystal structure of BCP will be a foundation on understanding more the mechanism of Mg substitution in BCP. Still, there are not many studies reporting the substitution effects on the crystal structure in BCP bodies (Gomes *et al.*, 2009; Kannan *et al.*, 2005). The structural representations of Mg-substituted BCP were modelled using VESTA software. Several lattices data from Rietveld refinement XRD analysis were required to generate crystal structure using this software. Studies regarding the generation of BCP crystal structure using this software is rarely being reported despite of some research works not involving BCP ceramics has reported the utilization of this tool (Haefeker *et al.*, 2014; Ohwada *et al.*, 2013; Skjærvø *et al.*, 2016).

Therefore, the purpose of this study is to synthesize Mg-substituted BCP bioceramic with varying Ca/P and Mg/Ca ratio through precipitation method as well as to study the low calcination temperature (500 – 600 °C) of BCP to understand the phase changes. The study of low calcination temperature of BCP would help us to control the final phase composition. A crystal structure of Mg-substituted BCP also expected to be generated using VESTA software.

1.3 Research objectives

There are three objectives that have to be fulfilled. The objectives are:

a) To synthesize Mg-substituted BCP bioceramics with different M/P (M = Ca + Mg) and Mg/Ca ratio through precipitation method.
b) To determine the effect of low temperature calcination of Mg-substituted BCP.
c) To construct the crystal structure modeling of Mg-substituted BCP using VESTA software.

1.4 Scope of research

Figure 1.1 shows the flowchart of the process involved in this work. This study involves four parts. Part 1 involves the synthesis of without Mg and Mg-substituted BCP powder through precipitation with different M/P and Mg/Ca ratio. This part considers various processes starting from mixing, aging, decantation, centrifugation, filtration, drying, and grinding; followed by the as-synthesized sample of without Mg and Mg-substituted BCP sample characterization in part 2 including thermogravimetric analysis (TGA), X-ray diffraction analysis (XRD), Fourier transform infrared spectroscopy (FTIR), inductively coupled plasma – atomic emission spectroscopy (ICP-AES), scanning electron microscopy (SEM), and energy dispersive X-ray spectroscopy (EDX) . Part 3 is the calcination process of both without substituted and Mg-substituted BCP powder at 500 and 600 °C, and finally their characterization in Part 4 which includes XRD, FTIR, ICP-AES, SEM, EDX, and transmission electron microscopy (TEM).

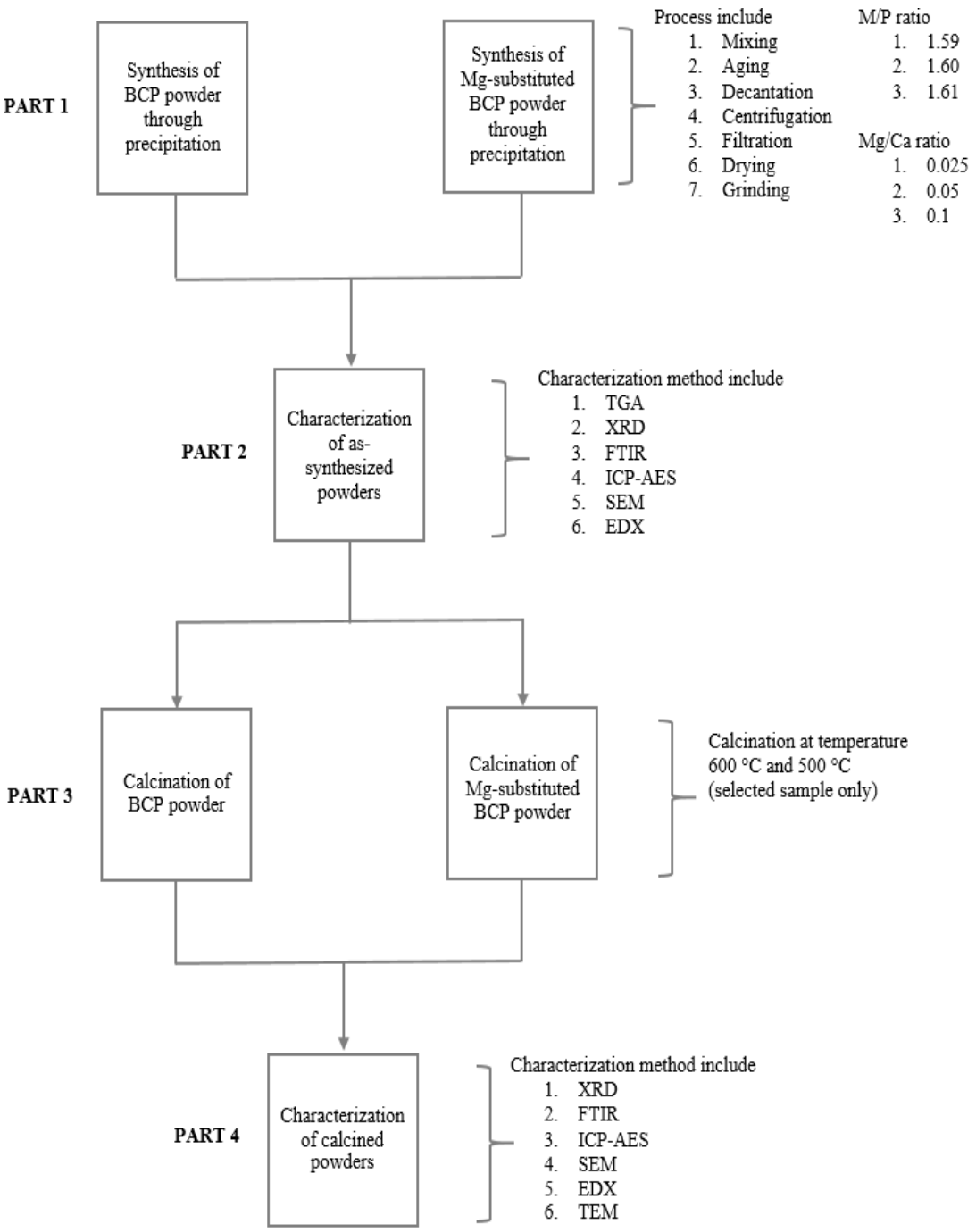

Figure 1.1 Flowchart of the research work

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

In the early application of bone grafting, it was considered as a trivial application to bond fractured bones together. The graft is a tissue or materials that can be used to repair a defect or deficiency in a biological system. Some of the grafts known in the bone system are autograft, allograft, xenograft, and alloplast. To enable a type of materials to be considered as a bone graft, it should fulfill several characteristics. The first characteristic is it should be readily available and not require surgical intervention at a second donor site. Secondly, it should not provide rapid osteogenesis and elicit immunological responses. Thirdly, it should be highly osteoinductive as this characteristic will provide for osteoconduction. Lastly, the bone graft material should help the formation of new attachment in periodontal lesions; not impeding bone growth (Janicki & Schmidmaier, 2011).

When considering bone graft materials, five physiologic considerations and process are being taken into consideration. The first physiologic considerations are osteoinduction; the induction of bone formation in the absence of a bony host site, followed by osteostimulation; a physiologic action that stimulates, enhances or accelerates the formation of bone at a host site. The third physiologic consideration is osteoconduction; a process in which synthetic and inorganic material provides a bioinert scaffolding that conducts bone growth, followed by osteopromotive; physical means of sealing off an anatomical site in order to prevent other tissues to interfere with bone formation, and finally osteogenesis; an action that occurs when a living osteoblasts are part of the bone grafts (Fernández *et al.*, 2015). The advancement in materials science that occurred presently, especially in the field of biomaterials, have provided so many

choices for synthetic bone scaffold either in the form of metals, polymers, ceramics or composite materials (Polo-Corrales *et al.*, 2014).

Synthetic scaffold (alloplast) has the advantage that its structures and materials properties can be tailored manually. One of the most potential materials for this purpose is bioceramics (Ducheyne, 2011). Some of the famous examples of bioceramics materials are glasses (bioglass), glass ceramics and ceramic filled bioactive composite; which can be tailored in porous or bulk form. The demand for bioceramics in the biomedical application has captured the interest of researchers in the study of calcium phosphates (CaP) materials for tissue engineering (Ducheyne, 2011). CaP has the same chemical composition that majorly occur in human bone (Dorozhkin & Epple, 2002). Synthetic CaP has been developed since then as a scaffold for bone regeneration. It is required that these scaffolds have high porosity to induce osteoconduction and sufficient strength as scaffold for bone growth. There are various preparation method in obtaining CaP bioceramics such as sol-gel, precipitation or through hydrothermal reaction (Xu *et al.*, 2013). The temperature required for the formation of different phases of CaP differs according to its preparation method. For an example, high temperature is required for formation of HA phase when using precipitation method.

One disadvantage of certain type of CaP is that the rate of bone bonding after implantation is quite low, thus require more time for bone healing. An approach to fix this condition is through the modification of CaP structures. The synthetic CaP body may be chemically substituted with a small number of elements such as strontium (Sr), magnesium (Mg) and others, which can be found in real bone. The substitution action could probably affect the dissolution rate of CaP and enhance osteoconduction (Best *et al.*, 2008). The purpose of writing this chapter is to provide a brief understanding regarding bone composition, structure, and grafting. This chapter also elaborates about

the bone substitutes, thus delving deeper towards the understanding of the chosen bioceramics for this study which is biphasic calcium phosphate (BCP) bioceramics, magnesium (Mg) substituted BCP, the effect of calcium to phosphorus ratio (Ca/P) in BCP bodies and the effect of calcination on BCP during fabrication process.

2.2 Bone

Bones in our body are living tissue which has its own blood vessels and cells that helps them to grow and recondition itself when subjected to injuries. Bone also can be defined as a mineralized dense connective tissue including proteins and vitamins. The functions of bone system are to give the framework of the body, attaching muscles and tendons, permits movements of our body, manufacture blood cells, and protecting our internal organs (Murphy *et al.*, 2018). This subchapter seeks to enlighten us the basic knowledge of bone systems comprising the composition, structure and bone grafting.

2.2.1 Bone composition

Bone is made up of collagen, non-collagenous proteins, other organics, and water. 90% of bone's organic materials constitute of collagen type I; which is bone itself and dentine (Sasagawa *et al.*, 2006). Collagen type I is known to reinforce the bone matrix. The main elements in bone collagen are calcium and phosphorus with approximately 25 and 12 wt % respectively (Boskey & Gehron Robey, 2013). This type of collagen provides the elasticity to the tissue. However, there also exist minor traces of other elements that are also playing key roles in bone health. The list of these minor elements is included in Table 2.1.

The calcified cartilage of bone is made from the type II collagen; related for long bone growth which later eroded and replaced by bone. The presence of this collagen in bone cartilage are up to 60 – 90 %. Collagen type II supports the back, jaw and joints in the skeletal system. It serves important functions in cell signaling, metabolism, and mineralization (Boskey & Gehron Robey, 2013). The amount of type II collagen usually lows in osteoporotic bone. The water content in bone serves many functions to fill the pores, interactions with collagens, and binding to mineral crystals (Yoder *et al.*, 2012). The remaining portion of the bone would include cells such as osteoblasts, osteoclasts, osteocytes and bone lining cells.

Table 2.1 Mineral composition of bone (Bastian, 2001; Kehoe & Eng, 2008; Combes et al., 2016)

Element	**Chemical formula**	**Weight percentage (wt %)**
Calcium	Ca	25
Phosphorus	P	12
Magnesium	Mg	0.37
Potassium	K	0.7
Zinc	Zn	0.009
Copper	Cu	0.0005
Sodium	Na	0.53
Cobalt	Co	0.0000025
Iron	Fe	0.0076
Strontium	Sr	0.05
Carbonates	CO_3^{2-}	5.6

2.2.2 Bone structure

Generally there are five classes of bones; namely long bones, short bones, flat bones, irregular bones and sesamoid bone (refer Figure 2.1 for images of bones). For an instance, long bones are viewed as a hollow shaft which mainly composed of dense cortical (compact) bones, while the metaphysis and epiphysis are composed of cancellous (trabecular) bone (refer Figure 2.1 for metaphysis and epiphysis part of the bone). Metaphysis is the narrow portion of a long bine above the epiphysis while the epiphysis is the end part of a long bone. This network of cancellous is lighter and less dense than the compact bone (Bart, 2008). 80% of the adult human bone is made of cortical bone and the remaining 20% of it are cancellous bone (Kobayashi *et al.*, 2003). Both cortical and cancellous bone contains osteons. Osteons (refer Figure 2.2) in cortical bone exist in cylindrical structures consists of concentric layers or lamellae of compact bone tissue that surround a central canal, also known as Haversian canal. This canal contains bone's blood supplies. Table 2.2 provides the understanding on the example of bone with regards to their category.

The external surface of bones, other than at the joint surfaces, is covered with a highly vascular, fibrous membrane, called periosteum. It maintains the connection with the underlying compact bone by means of direct continuation of its fibers. Periosteum consists of layers of the outer dense fibrous connective tissue layer and an inner loosely arranged osteogenic, or bone producing layer. Periosteum serves its function for development and growth of bones, providing nutrition of the underlying bone tissue, regeneration of bones due to fractures, and attaching muscles, tendons, and ligaments to the bones (Currey, 2002).

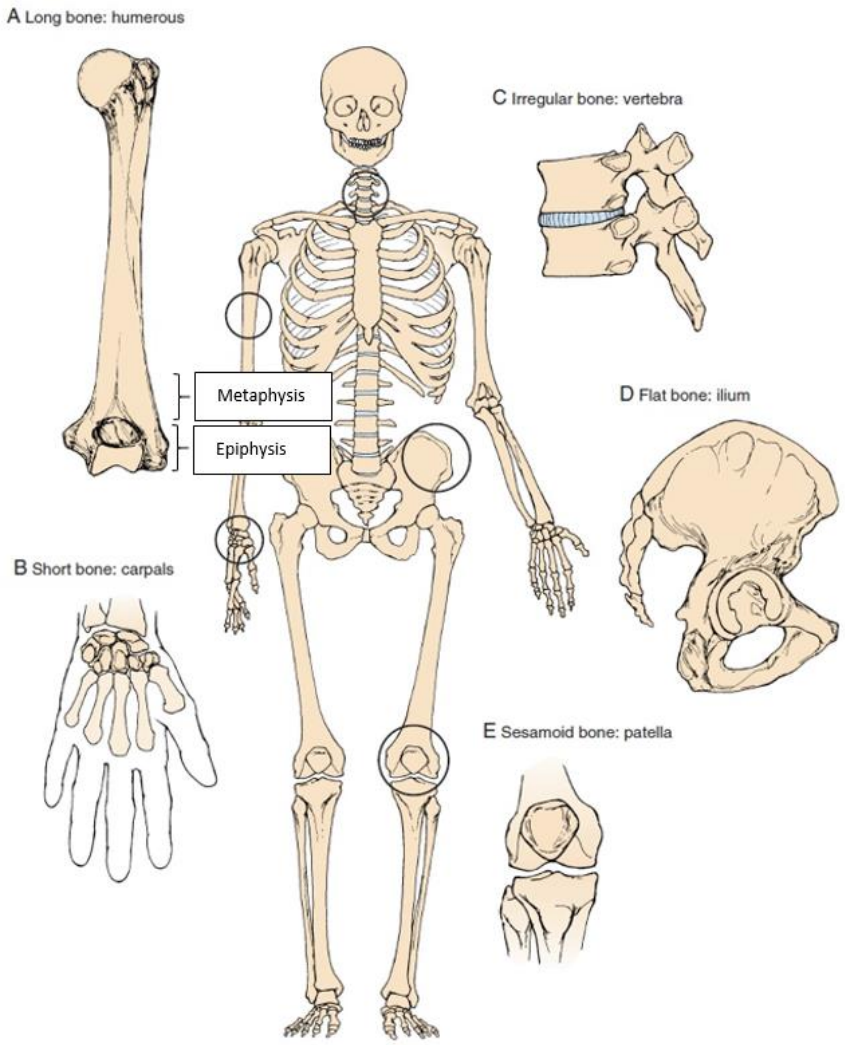

Figure 2.1 Images of bones in the human body (Premkumar, 2004)

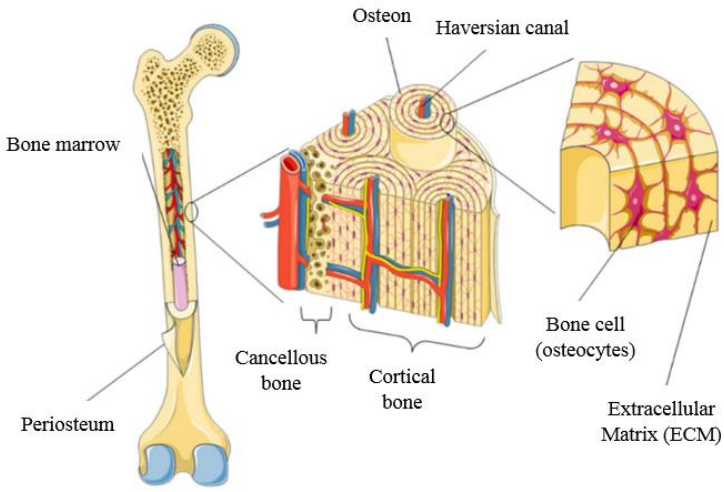

Figure 2.2 The structure of bone (Meng Bao et al., 2013)

Table 2.2 Category of bones and its examples (Clarke, 2008)

Category of bones	Examples
Long bones	Clavicles, humeri, radii, ulnae, metacarpals, femurs, tibiae, fibulae, metatarsals and phalanges
Short bones	Carpal and tarsal bones and sesamoid bones
Flat bones	Skull, mandible, scapulae, sternum, ilium, and ribs
Irregular bones	Vertebrae, sacrum, coccyx and hyoid bone
Sesamoid bone	Patella

2.3 Bone grafting

Bone grafting; or known as transplanting bone tissue, is a surgical procedure for fixing problem bones due to trauma or injuries. A bone graft functioned as structural support of articular fracture, fill the void to prevent fracture, and improved healing of fracture (Kumar *et al.*, 2013; Kuo *et al.*, 2013). The brief explanation of bone growth concerning osteoconduction, osteoinduction and osteogenesis have been discussed earlier in the subchapter 2.1 along with the type of bone graft. Table 2.3 provides the summary on the materials of bone substitutes and their respective properties. It can be seen from the table that autograft and allograft possess the characteristic for an ideal bone graft.

Table 2.3 Examples of bone substitute and their physiological properties

Source	Material	Osteocon-ductive	Osteoin-ductive
Alloplast	Polymer, ceramics, metals	Yes	No
Xenograft	Bovine bone, natural coral	Yes	No
Allograft	Harvested from one individual and transplanted into another within the same species	Yes	Yes/No
Autograft	Bone tissue transferred from one site to another in the same individual	Yes	Yes

2.3.1 Alloplast

Alloplast is one of the alternatives being used in bone repair materials other than xenograft, allograft, or autograft. It is also being known as synthetic bone substitutes. In order to be used in the biological system, alloplast need to have certain criteria such as biocompatible with host tissues, non-antigenic, non-carcinogenic, and non-inflammatory. Apart from that, they should exist in an ideally porous structure to allow tissue growth on them, stimulate bone induction, bioresorbable, and can be replaced by bone (Mah *et al.*, 2004).

Table 2.4 Currently available alloplast bone grafting materials approved by the Food and Drug Administration (FDA) (Mah et al., 2004)

Alloplast	**Brands**
Synthetic hydroxyapatite ceramics	Calcitite HA 2040 and 4060 (Sulzer Medica, Silzer Calcitek Inc., Carlsbad, CA 92008, USA)
	Osteogen (Implandent Ltd., Holliswood, NY, 11423, USA)
	Osteograf D (CeraMed Dental, Lakewood, CO 80028, USA)
Naturally derived hydroxyapatite ceramics	Interpore 200 (manufactured by Interpore International, distributed by Steri-Oss, Yorba-Linda, CA 92887, USA)
	Osteograf N (CeraMed Dental)
Calcium carbonate	BioCoral (INOTEB, Gonnery, France)
	Pro-Osteon 500R (Interpore Cross International, Irvine, CA 92618, USA)
Biocompatible composite polymer	Bioplant HTR (Septodont Inc., New Castle, DE 19720, USA)
Bioactive glass	Bioglass (registered trademark of US Biomaterials Corp. but licensed to Block Drug Corp., Jersey City, NJ 07302, USA
	Biogram (Orthovita, Malvern, PA 19355, USA)

Alloplast may be made from calcium phosphates and some of its famous examples are HA, bioactive glass, and biocompatible composite polymer. HA is commonly used due to its osteoconduction, hardness and acceptability by bone (Prants *et al.*, 2010). There are a few advantages of using alloplast compared to other bone grafts which are the unlimited availability (does not required donor patient), unlimited durability, no transfer of pathogens, and no immune reaction. However, there are also some disadvantages of this material which are less osteogenesis, less osteoinduction, debatable osteoconduction, uncertain absorption and transformation rates, and risk of infection with missing bony insertions or build up.

2.3.2 Xenograft

Xenografts are bone grafts that are derived from bovine, coral, and other sources than human, and be used as calcified matrix. Among the examples of xenograft are calf bone, keel bone, and anorganic bone. Table 2.5 shows the type and example of commercialized xenografts. Although having unlimited availability because it does not require a donor patient, xenograft, however, could introduce the risk of rejection and infections due to the presence of pathogens (normally occurred when xenograft being derived from animal source) (Figueiredo *et al.*, 2010).

Table 2.5 Commercially available xenograft bone grafting materials

Xenograft	**Brands**
Bovine bone	Bio-Oss (Geistlich Pharma North America Inc., 202 Carnegie Center Princeton, 08540 NJ, USA)
	Bio-Oss Collagen (Osteohealth Co., Shirley, NY, USA)
	Pepgen-P15 (Dentsply Sirona Inc., USA)
Porcine derived	OsteoBiol (Tecnoss)

2.3.3 Allograft

Allografts are grafts transferred between two same species donor and recipient. There are three types of allografts used in medical applications. They are fresh frozen bone, freeze-dried bone, and demineralized freeze-dried bone (Shibuya & Jupiter, 2015). Today, the demand for allogeneic bone grafts has led to the establishment of bone banking (de Alencar & Vieira, 2010). The purpose of bone banks is to preserve the integrity of the graft and the inductive protein of the bones, reducing its immunogenicity, and to ensure sterility. The acceptance of tissue donors for the bone banks begin with social and medical history. Donors are being tested positive or negative for diseases such as human immunodeficiency virus (HIV), hepatitis B, or another occult disease (Jackson, 1987).

Several advantages when opting to use xenograft as bone grafts are its adequate availability and elimination of an additional donor site surgery. However, some of its disadvantages are host incompatibility (risk of rejection by the body) and high risk of contamination of the graft. Another disadvantages is the process of obtaining allograft is complicated as it requires a number of procedure, which brings a lot of concerns medically, ethically, and legally (Poinern *et al.*, 2014).

2.3.4 Autograft

Autograft is widely used in periodontics for treatment of bony defects. This bone graft is obtained from the part of the patients' own body to eliminate the absence of crucial elements that required for bone formation and growth, which are osteogenesis, osteoinductive, and osteoconductive (Bellucci *et al.*, 2011). Autograft can be harvested from either intraoral or extraoral donor sites. One of the bone grafts were harvested from intraoral cancellous bone marrow for transplants (refer Figure 2.2 for the image of

cancellous bone marrow). One of the examples of extraoral bone grafts is hip marrow graft (iliac crest marrow) in the treatment of intrabony defects. However, autograft is not being recommended since last decade when involving large or multiple defects due to the morbidity of the donor site, post operation impact, exfoliation and sequestration, rapid reoccurrence of the defect, and patient's expenses and difficulty (Yasukawa *et al.*, 2004).

2.4 Biomaterials

Biomaterials is a synthetic material that could replace a part or a function of the body in a safe, reliable, economic and physiologically acceptable manner (Bronzino, 2000). It should have the ability to be in contact with tissue, blood, and biological fluids. It is meant to be used as prosthetic, diagnostic, therapeutic, and storage applications without affecting our body and organs. Alloplast is considered as one type of biomaterials. There are two other important terms in defining biomaterials; which are biological materials, means a material that is produced by the biological system, and biocompatibility means acceptance of an artificial implant by the surrounding tissues and the body as a whole (Anderson, 2016).

There are several properties of biomaterials need to be taken into account for its implementations in biological systems. First, the mechanical properties of the biomaterials. Second, the chemical properties, which includes the solubility and erosion, and corrosion behavior. Ideally, biomaterials should fit the demanded biomaterials properties that would suit its applications. Biomaterials have been used widely for replacement of diseased and damaged parts, like the artificial hip joint, assisting in the healing process, like the sutures, bone plate, and screws, improving the

function of an organ, like the cardiac pacemaker, or aiding in the treatment process, like the catheters and drains (Jaganathan *et al.*, 2014).

Materials ranging from polymers, metals, ceramics, and composites has been implemented as biomaterials. Hence, it is crucial to acknowledge the science of biomedical materials, which are the composition, properties of materials, and the way they interact with the body environment. An assorted amount of polymeric materials have been used as biomaterials, for examples, acrylics, polyamides, polyesters, polyethylene and more. Some of the typical applications for polymeric biomaterials include artificial heart, kidney, liver, pancreas, catheters, and contact lenses (Dumitriu & Popa, 2013). For the applications of metals in biomaterials, the most frequently used metallic system in the body are iron-based alloys of 316L stainless steel, titanium and titanium-based alloys, and cobalt-based alloys (Helsen & Breme, 1998).

Composite biomaterials have been implemented widely in dentistry and prosthesis designers (Ambrosio, 2009). One of the famous examples is the ultrahigh-molecular-weight polyethylene (UHMWPE) reinforced with carbon fibers. It is being used in the design of prostheses. Composites are unique and usually stronger than any single materials. Most frequently used biomaterial ceramics or bioceramics are aluminum oxides, calcium phosphates, apatites, graphite, and glass (Kokubo, 2008). Ceramics have been used in the biological system due to its inertness in the body, its high compressive strength, and in some cases, its excellent wear characteristics. They are used in the applications of hip prostheses, artificial knees, bone grafts (allografts) and tissue growth applications as in orthopedics, dentistry and heart valves.

2.5 Bioceramics

Ceramic materials that are used for repair and replace damaged parts of the skeletal system is referred to as bioceramics (Amini *et al.*, 2012). It has been widely used in the applications of orthopedic load-bearing coatings, dental implants, bone graft substitutes, and bone cement. The three common types of bioceramics; bioinert, bioactive and bioresorbable can be produced in crystalline and amorphous forms (Kutz, 2003).

Bioinert bioceramics have the ability to maintain their physical and mechanical properties while in the host. They are typically used as structural-support implants such as bone plates, bone screw, and femoral heads. One of the famous examples of this type of bioceramics is alumina (Al_2O_3); which has been widely used in the femoral head of a hip implant and implant coating (Lee, 2016). Bioactive bioceramics provide direct and strong chemical bond with tissues and it is used for fixation of implants in the skeletal system. Among the examples of them would be glass ceramics and dense nonporous glasses. Figure 2.3 shows the uses of bioceramics on several types of bones. Meanwhile, bioresorbable bioceramics functioned by chemically broken down by the body, degrade and be replace by new forming tissue. For this to happen, the chemical produced by the bioceramics as it is being resorbed must be able to be processed by the body without being rejected. Some of the examples of bioresorbable bioceramics are CaP, calcium sulfate, HA, TCP, ferric-calcium-phosphors oxides and corals (Eliaz & Metoki, 2017).

Bioceramics are conventionally made by various popular method including sol-gel and precipitation technique (Dong Hyun *et al.*, 2015; Marzban & Mahmood Rabiee, 2017). The term ‘sol’ denotes the stable colloidal solution, while the term ‘gel’ is the self-supportable hardened body obtained from sol (Kokubo, 2008). This method can also be used to process glass-ceramics biomaterials. The latter process, precipitation,

was known to be low cost and being applied commonly in the industrial production of bioceramics. CaP, HA, TCP bioceramics are usually produced using this method. Precipitation method usually results in small size and low crystallinity of end products. However, this process highly depends on the synthesis condition and also the pH value as it involves liquid to solid phase transition (Dong Hyun *et al.*, 2015). It should be noted that both methods is namely called wet-chemical as they use solutions as starting sources. This method has been widely used to manufacture crystalline bioceramics due to its high homogeneity at a relatively low temperature and also the mixture of liquid sources ensures that elements are mixed at the atomic level (Kokubo, 2008).

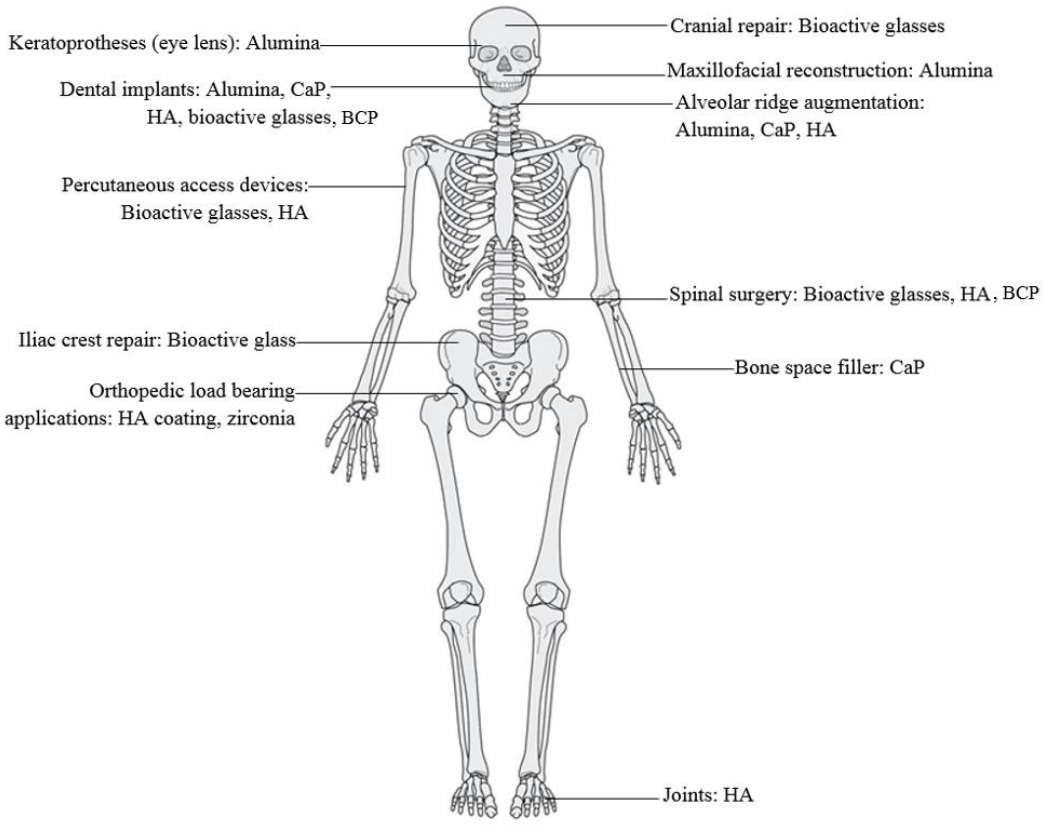

Figure 2.3 Bone parts and their respective biomaterials implementations

2.6 Calcium phosphate (CaP) bone substitutes

CaP based bioceramics have been proven to benefit the biomedical fields, especially in bone defect repair. CaP is a family of materials and minerals containing calcium ions (Ca^{2+}) and phosphate ions ($(PO_4)^{3-}$). Some of it also contains traces of oxide and hydroxide too. The basic chemical formula for CaP is $Ca_{10}(PO_4)_6X_2$; where X could be fluoride (F^-), chloride (Cl^-) or hydroxide (OH^-). CaP that exists in the form of hydroxyapatite (HA) present in bone, teeth, and tendons; giving them stability, hardness and functions (Eliaz & Metoki, 2017). Apart from its existence in the human body, calcium phosphates also exist in geological environments where it exists as a mixture of other elements. Strontium (Sr), barium (Ba) and magnesium (Mg) are some of the impurities that exist in naturally occur CaP due to the ability of calcium ions to be replaced by these ions, while phosphate ions normally being replaced by carbonate (CO_3^{2-}) ions (Dorozhkin & Epple, 2002). The reason why this type of bioceramic is suitable for repairing damaged bone tissue is solely due to the chemical and structural similarity to our bone mineral component as shown in Figure 2.1 (Stipniece, 2016).

CaP comes in a variety of phases. The most important parameter to distinguish these CaP phases are the Ca/P molar ratio and solubility. Table 2.6 shows the summarization of the CaP compound and their solubility product constant (log K_{sp}). Ca/P molar ratio will denote the deficiency of calcium in the CaP bioceramics while the solubility of Ca/P indicate its reactivity with solutions in human body. In general, lower Ca/P ratio denotes that the CaP is more acidic and more soluble in water, thus easily disintegrates in human body (Koutsoukos *et al.*, 1980).

The properties of CaP are strongly dependent on the Ca/P ratio. When the Ca/P ratio is below 1.67, the material becomes less stable thus causes the dissolution rate to become faster hence leading to mechanical failure of the implant *(*Prezas *et al.*, 2017).

Two of the most common CaP bioceramics known are HA and β-TCP. As shown in Table 2.6, the Ca/P ratio of both HA and β-TCP phase are 1.67 and 1.5 respectively. When Ca/P ratio is in between 1.5 and 1.67 (1.5 < Ca/P < 1.67), it correspond to the formation of BCP with the chemical formulation of $Ca_{10-x}(PO_4)_{6-x}(HPO_4)_x(OH)_{2-x}$.

Table 2.6 Example of CaP compound and their solubility product constants (Kokubo, 2008)

Compound/phase	**Abbreviation**	**Chemical formula**	**Ca/P**	**log K_{sp}**
Monocalcium phosphate monohydrate	MCPM	$Ca(H_2PO_4)_2.H_2O$	0.5	Highly soluble
Monocalcium phosphate anhydrous	MCPA	$Ca(H_2PO_4)_2$	0.5	Highly soluble
Dicalcium phosphate dihydrate	DCPD	$CaHPO_4.2H_2O$	1.0	6.59
Dicalcium phosphate anhydrous	DCPA	$CaHPO_4$	1.0	96.6
Octacalocium phosphate	OCP	$Ca_8H_2(PO_4)_6.5H_2O$	1.33	96.6
alpha-tricalcium phosphate	α-TCP	$Ca_3(PO_4)_2$	1.5	
beta-tricalcium phosphate	β-TCP	$Ca_3(PO_4)_2$	1.5	28.9
Hydroxyapatite	HA	$Ca_{10}(PO_4)_6(OH)_2$	1.67	116.8
Fluoroapatite	FAP	$Ca_{10}(PO_4)_6F_2$	1.67	121
Tetracalcium phosphate	TTCP	$Ca_4(PO_4)_2O$	2.0	38-44

CaP could be used as a bone substitution in cement form (known as calcium phosphate cement, CPC). CPC consists of one or more CaP powders, which harden upon mixing with water or an aqueous solution, forming either HA or DCPD phases. Various other type of CaP compound also can be used as CPC. Few properties such as setting time, cohesion and anti-washout ability, and injectability are needed to be taken into consideration when using this product (Zhang *et al.*, 2014). It is required that CPC

has fast setting time after application. The smaller particle size and low crystallinity of CPC have known to reduce the setting time. Cohesion is the ability of a mixture to harden in a static aqueous environment. Bad cohesion preventing the mixture or paste to setting thus leading to negative in vivo reactions due to the release of microparticles.

Injectability is the ability for a formulation to be extruded through a syringe. Among the factors which could increase injectability are smaller particle size and a decrease in viscosity (Dorozhkin, 2013). Apart from these properties, mechanical properties such as strength fracture toughness are crucial in the application of CPCs. To increase these mechanical properties, reinforcement such as carbon nanotubes, apatite seeds, $CaCO_3$, citric acid and sodium citrate, and fiber has been introduced in CPC. Bioresorption is also one of the critical properties that CPC should have. For an instance, for MCPM, MCPA, DCPD and DCPA, the bioresorbability of these product are several times higher than α-TCP, β-TCP, BCP, HA and TTCP, thus making them more soluble and physically dissolve faster in vivo (Zhang *et al.*, 2014).

Reynaud *et al.* reported that Ca/P ratio affect the surface area of its powder end products where there is a decrement in CaP powder surface area when the Ca/P ratio is high. The increment in Ca/P ratio can be obtained when CaP powders were subjected to heat treatment. This action activates particle growth thus resulting in a decrement of surface area. The next subchapters will highlight more on the bioceramics involved in this study which are HA, β-TCP and BCP.

2.6.1 Hydroxyapatite (HA)

Since BCP is the main interest in this study, it is presentable to enlightens regarding two of the main phase exist, which is HA and β-TCP. This subchapter will give explanation concerning the HA phase. Calcium hydroxyapatite, or known as HA