SCHOOL OF MATERIALS AND MINERAL RESOURCES ENGINEERING

UNIVERSITI SAINS MALAYSIA

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

By

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Dissertation submitted in partial fulfillment of the requirements for the degree of Bachelor of Engineering with Honours (Materials Engineering)

Universiti Sains Malaysia

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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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<td>TCP</td>
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<td>TE</td>
<td>Tissue Engineering</td>
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<td>TG</td>
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<td>XRD</td>
<td>X-ray Diffraction</td>
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LIST OF SYMBOLS

* Degree

\( \theta \)  Theta

\%Vs  Percentage of shrinkage in volume

\%T  Percentage of Transmittance

\( \rho_{\text{bulk}} \)  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 Jenamaan 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. Walau bagaimanapun, 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 penghasilan 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\textsuperscript{2+}) SUBSTITUTION AND CALCINATION TO THE PROPERTIES OF BIPHASIC CALCICUM 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\textsuperscript{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\textsuperscript{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\textsuperscript{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 ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) 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 ($\text{Ca}_3(\text{PO}_4)_2$) is a bioactive and biodegradable material. TCP has four polymorphs: $\beta$, $\alpha$, $\alpha'$ and $\gamma$ phase. Each phase has different stable temperature. Among the four polymorphs of TCP, $\beta$-TCP is investigated intensively as a bioactive bone graft material because it needs low temperature to prepare and does not have an issue with high reactivity (Eliaz & Metoki, 2017).

HA and $\beta$-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 $\beta$-TCP are considered better when compared with either single-phase HA or $\beta$-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), Ca₃(PO₄)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 like 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).
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.
The main elements in bone mineral are calcium and phosphorus with 25.4 wt% and 11.6 wt% composition, respectively. There is also a 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)

<table>
<thead>
<tr>
<th>Elements</th>
<th>Chemical Formula</th>
<th>Bone composition (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Major Elements</td>
</tr>
<tr>
<td>Calcium</td>
<td>Ca</td>
<td>25.4</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>P</td>
<td>11.6</td>
</tr>
<tr>
<td>Carbonates</td>
<td>( \text{CO}_3^{2-} )</td>
<td>5.6</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>N</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Minor Elements</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Mg</td>
<td>0.27</td>
</tr>
<tr>
<td>Sodium</td>
<td>Na</td>
<td>0.53</td>
</tr>
<tr>
<td>Chloride</td>
<td>Cl</td>
<td>0.13</td>
</tr>
<tr>
<td>Potassium</td>
<td>K</td>
<td>0.0047</td>
</tr>
<tr>
<td>Sulphide</td>
<td>( \text{S}^{2-} )</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Main Trace Elements</td>
</tr>
<tr>
<td>Cobalt</td>
<td>Co</td>
<td>0 - 2.5×10^{-6}</td>
</tr>
<tr>
<td>Zinc</td>
<td>Zn</td>
<td>0 – 3.9×10^{-3}</td>
</tr>
<tr>
<td>Strontium</td>
<td>Sr</td>
<td>0 – 0.05</td>
</tr>
<tr>
<td>Iron</td>
<td>Fe</td>
<td>7.6×10^{-3}</td>
</tr>
<tr>
<td>Fluoride</td>
<td>F</td>
<td>0.04</td>
</tr>
<tr>
<td>Aluminium</td>
<td>Al</td>
<td>2.9×10^{-3}</td>
</tr>
<tr>
<td>Lead</td>
<td>Pb</td>
<td>4.4×10^{-4}</td>
</tr>
</tbody>
</table>
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)

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

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)

<table>
<thead>
<tr>
<th>Classification</th>
<th>Tissue Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxic material</td>
<td>Tissue dies</td>
</tr>
<tr>
<td>Bioinert material</td>
<td>Tissue form an adherent fibrous capsule around the implant</td>
</tr>
<tr>
<td>Bioactive material</td>
<td>Tissue for interfacial bond with implant</td>
</tr>
<tr>
<td>Bioresorbable material</td>
<td>Tissue eventually replace implant as new bone formation take place</td>
</tr>
</tbody>
</table>

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)

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

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$_2$O$_3$) 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$_2$O). 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$_2$H$_2$(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>
<thead>
<tr>
<th>Compound name</th>
<th>Abbreviation</th>
<th>Formula</th>
<th>Ca/P ratio</th>
<th>pH stability</th>
<th>Crystal structure</th>
<th>Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocalcium phosphate monohydrate</td>
<td>MCPM</td>
<td>Ca(H$_2$PO$_4$)$_2$.H$_2$O</td>
<td>0.5</td>
<td>0.0 - 2.0</td>
<td>Triclinic</td>
<td>2.23</td>
</tr>
<tr>
<td>Monocalcium phosphate anhydrate</td>
<td>MCPA</td>
<td>Ca(H$_2$PO$_4$)$_2$</td>
<td>0.5</td>
<td>[a]</td>
<td>Triclinic</td>
<td>2.58</td>
</tr>
<tr>
<td>Dicalcium phosphate dehydrate (brushite)</td>
<td>DCPD</td>
<td>CaHPO$_4$.2H$_2$O</td>
<td>1.0</td>
<td>2.0 - 6.0</td>
<td>Monoclinic</td>
<td>2.32</td>
</tr>
<tr>
<td>Dicalcium phosphate anhydrate (monetite)</td>
<td>DCPA</td>
<td>CaHPO$_4$</td>
<td>1.0</td>
<td>[a]</td>
<td>Triclinic</td>
<td>2.89</td>
</tr>
<tr>
<td>Octacalcium phosphate</td>
<td>OCP</td>
<td>Ca$_8$(HPO$_4$)$_2$(PO$_4$)$_4$.5H$_2$O</td>
<td>1.33</td>
<td>5.5 - 7.0</td>
<td>Triclinic</td>
<td>2.61</td>
</tr>
<tr>
<td>α-tricalcium phosphate</td>
<td>α-TCP</td>
<td>α-Ca$_3$(PO$_4$)$_2$</td>
<td>1.5</td>
<td>[b]</td>
<td>Monoclinic</td>
<td>2.86</td>
</tr>
<tr>
<td>β-tricalcium phosphate</td>
<td>β-TCP</td>
<td>β-Ca$_3$(PO$_4$)$_2$</td>
<td>1.5</td>
<td>[b]</td>
<td>Rhombohedral</td>
<td>3.07</td>
</tr>
<tr>
<td>Amorphous calcium phosphate</td>
<td>ACP</td>
<td>Ca$_x$(PO$_4$)$_y$.nH$_2$O</td>
<td>1.2 – 2.2</td>
<td>[e]</td>
<td>Hexagonal</td>
<td>1.75</td>
</tr>
<tr>
<td>Calcium-deficient hydroxyapatite</td>
<td>CDHA</td>
<td>Ca$_{10-x}$(HPO$_4$)$_x$(PO$_4$)$_6-x$(OH)$_2$$_x$ (0&lt;$x$&lt;1)</td>
<td>1.5 - 1.67</td>
<td>6.5 - 9.5</td>
<td>Hexagonal</td>
<td>1.59</td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>HA</td>
<td>Ca$_{10}$(PO$_4$)$_6$(OH)$_2$</td>
<td>1.67</td>
<td>9.5 - 12</td>
<td>Monoclinic or hexagonal</td>
<td>3.16</td>
</tr>
<tr>
<td>Tetracalcium phosphate</td>
<td>TTCP</td>
<td>Ca$_4$(PO$_4$)$_2$O</td>
<td>2.0</td>
<td>[b]</td>
<td>Monoclinic</td>
<td>3.05</td>
</tr>
</tbody>
</table>

[a] Stable at temperature above 100 °C. [b] Cannot be precipitated. [c] Always metastable
Hydroxyapatite (HA) is one of the members in the apatite group ceramics which is the main constituent of the inorganic part of the bone structure with a stoichiometric Ca/P ratio of 1.67 (Sibte et al., 2013). HA has chemical formula \( \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \) (Gonzalez Ocampo et al., 2016).

Over the years, HA has been recognized as an excellent biomaterial since HA has widely been used in bone reconstructive surgery due to outstanding biological compatibility 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
applications (Al-Sanabani et al., 2013). HA has two different crystals structure which are monoclinic and hexagonal. At the temperature above 250 °C, there is monoclinic to hexagonal phase transition in HA. The two structures have firmly like the mineral phase of the human bone. Monoclinic crystal structure can only be obtained at high temperatures but it most ordered and thermodynamically stable form if compared to hexagonal structure (Uysal et al., 2014). In opposite site, hexagonal structure is the most stable phase of various calcium phosphates. It is stable in body fluid and in dry or moist air up to 1200 °C and does not decompose. HA has appeared to be bioactive material because of its resorbable behaviour (Thamaraiselvi & Rajeswari, 2004). Calcination temperature and duration time are crucial stages for producing HA due to various mechanical properties at various ranges (Uysal et al., 2014). Thus, appropriate step of producing HA should be considered in order to obtain its desired properties.

2.6.2.1 Limitation of Hydroxyapatite (HA)

Through all advantages, HA also has suffered some disadvantages to be utilized for long-term biomedical application due its possess slow osteointegration rate (Thian et al., 2013). Even though HA has great biocompatible and osteoconductive properties, its suffer from limited bioactivity (Kumar et al., 2012). This limitations happens because of its stability that leads to extremely slow degradation rate in biological fluids, unseemly degradation rate cause neglect to complement in vivo bone regeneration (Mickiewicz, 2001).

Also, poor osteogenic and angiogenic properties in HA will lead to less feasibility of tissue at the site of bone grafting. Both osteogenic and angiogenic properties are crucial for biomaterials designed as bone implants because bone formation needs bone growth factor as well as blood vessel growth factor (Kulanthaivel et al., 2015). Figure 2.5 shows
the process of long bone development which undergo both osteogenesis and angiogenesis process (Egawa et al., 2014).

Figure 2.5: Long bone development which undergo both osteogenesis and angiogenesis process (Egawa et al., 2014)

2.6.3 Beta Tricalcium Phosphate (β-TCP)

The chemical formula of tricalcium phosphate (TCP) is Ca₃(PO₄)₂. TCP has four polymorphs: α, β, γ and α’. The γ-polymorph phase is a high-pressure phase, and the α’ polymorph phase is observed at temperature above approximate 1500 °C (Kannan et al., 2007). The process of phase transformation involves gradual reduction in density upon treated with high temperature (Ryu et al., 2002). Therefore, the most frequently observed TCP polymorphs in the field of bioceramics are α-TCP and β-TCP.

Beta-tricalcium phosphate (β-TCP) is the low-temperature phase in the CaO-P₂O₅ phase diagram. β-TCP normally formed through heating at temperature below than 1150
°C. As at temperature higher than that, alpha-tricalcium phosphate (α-TCP) phase formation will occur. Both β-TCP and α-TCP are having same chemical composition, but β-TCP has different in density, chemical structure and solubility level as compared with α-TCP (Li et al., 2016). Different in properties will also determine their different in biological compatibility also medical application properties applied to produce bioceramics that able to degrade with high density (Hurle et al., 2017).

Essentially, high biodegradation rate of β-TCP is an ideal implant material (Sunarso, 2013). After implantation, β-TCP able to react well with other surrounding host tissue by formation of direct bond due to great biological performance. Then, β-TCP will degrade with time and be replaced with natural tissues. It leads to the regeneration of tissue instead of their replacement and thus solves the problem of interfacial stability (Eliaz & Metoki, 2017).

However, in order to use for surgical implantation, the mechanical performance of an implant must match the repair rate of body tissues, thus the biodegradation rate of the implant must be controlled very well. In order to solve this problem, a mixture between bioactive material and bioresorbable material was mixed together and for instance the biphasic calcium phosphate (BCP) between β-TCP and HA (Kannan et al., 2011).

2.7 Biphasic Calcium Phosphate (BCP)

Development of biphasic calcium phosphate (BCP), particularly with HA and β-TCP has drawn significant consideration. HA and β-TCP, in spite of the fact that have similar chemical composition, they contrast in their biological resorbing capacity. The HA ceramics, despite being considered as the ideal material for bone substitution due to its similitude with the bone mineral and osteoconductive property, it is non-resorbable
and bio-inert due to its less soluble nature in aqueous media, while β-TCP demonstrated rapid biodegradation. Consequently, a combination of HA with β-TCP is expected to induce bone growth by provide osteoconductive material with higher reactivity and improve the poor biodegradation characteristics of HA ceramics (Kannan et al., 2011).

2.7.1 Substitution of Magnesium Ion into Biphasic Calcium Phosphate (Mg-BCP)

Among substituting ions, magnesium is one of the studied ion and the fourth most abundant cation present in the human body. For example, in enamel contain 0.10-0.44 wt% of magnesium. In dentin, amount of magnesium around 1.10-1.30 wt% while in bone, there are range within 0.60-0.70 wt% of magnesium. Amount of magnesium in dentin and enamel will increase from the surface of enamel to the boundary enamel-dentin (Sader et al., 2009).

Magnesium is also one of the important ion that links with biological apatite. It has been confirmed that in hard tissues of calcium, the magnesium content that links with apatite phase is high at the beginning stage of the calcification process and decreases with the increase of calcification (Kannan et al., 2010). The lack of magnesium affects all stages of bone system metabolism. It can lead to inhibition of bone growth, lowering osteoblast activity and also bone fragility (Sader et al., 2009).

The modification of various biomaterials has been performed through incorporation of Mg$^{2+}$ into the crystal structure of component, in this case CaP. CaP with Mg$^{2+}$ incorporation has been the subject of extensive research because for a number of reasons: (a) better understanding of biomineralization processes, (b) increase of bioactivity and (c) localized targeted delivery of the ions able to act on bone disease such as osteoporosis. In addition, the design of biomaterials, which exploit Mg$^{2+}$ release ability for initiating bone-framing cells, is of great attention (Suchanek et al., 2004).
Various research groups have attempted to dope BCP with Mg\(^{2+}\). Doping of Mg\(^{2+}\) into BCP will result in biological and mechanical improvement as the ion will cause the acceleration of nucleation kinetics of bone minerals (Kannan et al., 2008). It is hypothesized that Mg-modified BCP bioceramic would ensure faster and more efficient recovery of damaged bone tissues by decomposing into environment of the body (Jang et al., 2015). Mg\(^{2+}\) is outstanding to encourage cellular adhesion onto the substrate, and is likewise known to improve proliferation, differentiation, calcification and angiogenic functions (Kumar et al., 2015).

2.7.2 Method for Synthesis BCP and Mg-BCP Based Powders

There are various methods used to prepare BCP and Mg-BCP powders. Every type of techniques for processing conditions were varied in a wide range, causing in several sub methods. Some modifications on these techniques also be done. In present, there are five groups of preparation techniques, which are high-temperature processes (with two subgroups), synthesis methods based on biogenic sources and combination procedures (Sadat-shojai et al., 2013). Summarization of these classes of methods are shown in Table 2.6.
Table 2.6: Type of methods used to synthesize BCP and Mg-BCP powders (Sadat-shojai et al., 2013)

<table>
<thead>
<tr>
<th>Method</th>
<th>Processing aspects</th>
<th>Characteristics of powder</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of chemicals</td>
<td>Cost</td>
</tr>
<tr>
<td>Dry methods</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solid-state method</td>
<td>Few</td>
<td>Low</td>
</tr>
<tr>
<td>Mechanochemical method</td>
<td>Few</td>
<td>Low</td>
</tr>
<tr>
<td>Wet methods</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemical precipitation</td>
<td>Frequently few</td>
<td>Low</td>
</tr>
<tr>
<td>Hydrolysis method</td>
<td>Few</td>
<td>Usually high</td>
</tr>
<tr>
<td>Sol-gel method</td>
<td>Variable</td>
<td>Variable (usually low)</td>
</tr>
<tr>
<td>Hydrothermal method</td>
<td>Variable</td>
<td>Usually high</td>
</tr>
<tr>
<td>Emulsion</td>
<td>Many</td>
<td>High</td>
</tr>
<tr>
<td>Sonochemical method</td>
<td>Few</td>
<td>Usually low</td>
</tr>
</tbody>
</table>
Table 2.6: Type of methods used to synthesize BCP and Mg-BCP powders (Sadat-shojai et al., 2013) (Continue)

<table>
<thead>
<tr>
<th>High temperature process</th>
<th>Combustion method</th>
<th>Few</th>
<th>Usually low</th>
<th>Diverse (usually irregular)</th>
<th>Variable</th>
<th>Usually high</th>
<th>Variable</th>
<th>Usually nano</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrolysis method</td>
<td>Variable</td>
<td>Usually low</td>
<td>Diverse</td>
<td>High</td>
<td>Variable</td>
<td>Usually stoichiometric</td>
<td>Nano particle embedded in micron aggregates</td>
<td></td>
</tr>
<tr>
<td>Synthesis from biogenic sources</td>
<td>Few</td>
<td>Usually low</td>
<td>Diverse</td>
<td>Variable</td>
<td>Usually high</td>
<td>Variable</td>
<td>Variable</td>
<td></td>
</tr>
<tr>
<td>Combination procedures</td>
<td>Variable</td>
<td>Variable</td>
<td>Diverse (frequently needle-like)</td>
<td>Frequently high</td>
<td>Usually high</td>
<td>Usually stoichiometric</td>
<td>Usually nano</td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 Introduction

Chapter 3 is focusing on the materials and methodology used during this research in order to fabricate biphasic calcium phosphate (BCP) and magnesium-doped biphasic calcium phosphate (Mg-BCP) pellets. In general, this chapter can be distributed into four main sections. The raw materials and preparation for synthesis of BCP and Mg-BCP powders will be discuss in Section 3.2. Section 3.3 explained about the characterization techniques that were performed on the as-synthesized BCP and Mg-BCP powders. For Section 3.4 and Section 3.5 will clarified about fabrication of BCP and Mg-BCP pellets and characterization techniques that were performed on its respectively. Figure 3.1 summarized the experiments involved in this research work.

In Section 3.3 and Section 3.5, there are characterization techniques can be used in order to characterize as-synthesized powders and calcined BCP and Mg-BCP pellets. Thermogravimetric-Differential Scanning Calorimetry (TG-DSC) and Fourier Transform Infrared Spectroscopy (FTIR) were used to understand the thermal behaviour and bonding function respectively. X-ray diffraction (XRD) was used to analysis the crystal structure, crystallinity and phase identification of pellets. Also, the morphology of pellets is determined by Field Emission Scanning Electron Microscopy (FESEM).

In Section 3.5, characterization is also important to examine the physical and mechanical properties of the calcined BCP and Mg-BCP pellets. The calcined BCP and Mg-BCP pellets were physically tested with shrinkage measurement, porosity and density measurement based on Archimedes principle. While, the calcined BCP and Mg-BCP pellets were tested with hardness test.

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Section 3.2

Synthesis of powders
- Wet precipitation method
- 1.5 of Ca/P molar ratio

Section 3.3

Characterization of as-synthesized powders
- Thermogravimetry (TG) and Differential Scanning Calorimetry (DSC)
- X-ray Diffraction (XRD)
- Fourier Transform Infrared Spectroscopy (FTIR)
- Scanning Electron Microscopy (SEM)

Section 3.4

Calcined pellet preparation
- 0.5g of powder for each pellet
- Pressing (50 MPa) for 2 minutes
- Calcination
  - Soaking time 120 minutes
  - Heating rate 5 °C/min
  - Temperature: 600 °C, 700 °C and 800 °C

Section 3.5

Characterization/Testing
- X-ray Diffraction (XRD)
- Scanning Electron Microscopy (SEM)
- Linear Shrinkage Measurement
- Bulk Density Measurement
- Hardness Test (Vickers)

General empirical formula for as-synthesized powder

**BCP:** \( \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \)

**Mg-BCP:** \( \text{Ca}_{10-x}(\text{HPO}_4)_x(\text{PO}_4)_{6-x}(\text{OH})_{2-x} \)

\( x = \text{Mg}^{2+} \) ions

Figure 3.1: Flowchart of overall research work
3.2 Synthesis of Biphasic Calcium Phosphate (BCP) and Magnesium-doped Biphasic Calcium Phosphate (Mg-BCP) Powders

3.2.1 Raw Materials

The raw materials that were used to synthesize BCP and Mg-BCP powders are listed in Table 3.1. Calcium nitrate tetrahydrate (Ca(NO$_3$)$_2$. 4H$_2$O) and di-ammonium hydrogen phosphate ((NH$_4$)$_2$HPO$_4$) were used as precursors for calcium ion (Ca$^{2+}$) and phosphate ion (PO$_4^{3-}$) respectively. Magnesium nitrate hexahydrate (Mg(NO$_3$)$_2$. 6H$_2$O) was used as sources of magnesium (Mg$^{2+}$) precursors. Ammonium hydroxide (NH$_4$OH) was used to control the pH value at 10-11 approximately.

Table 3.1: List of raw materials for synthesis and their function

<table>
<thead>
<tr>
<th>Material</th>
<th>Chemical Formula</th>
<th>Molecular Weight (g/mol)</th>
<th>Source/Manufacturer</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium nitrate tetrahydrate</td>
<td>Ca(NO$_3$)$_2$. 4H$_2$O</td>
<td>236.15</td>
<td>Merck (99.95 % pure)</td>
<td>Calcium precursor</td>
</tr>
<tr>
<td>Di-ammonium hydrogen phosphate</td>
<td>(NH$_4$)$_2$HPO$_4$</td>
<td>132.05</td>
<td>Merck (99.99 % pure)</td>
<td>Phosphate precursor</td>
</tr>
<tr>
<td>Magnesium nitrate hexahydrate</td>
<td>Mg(NO$_3$)$_2$. 6H$_2$O</td>
<td>256.41</td>
<td>Merck (99.99 % pure)</td>
<td>Magnesium precursor</td>
</tr>
<tr>
<td>Ammonium hydroxide</td>
<td>NH$_4$OH</td>
<td>35.04</td>
<td>Merck (99.99 % pure)</td>
<td>pH control</td>
</tr>
</tbody>
</table>
Table 3.2 shows the molar ratio of the starting raw materials for synthesis BCP and Mg-BCP powders in this research work.

Table 3.2: The molar ratio of the starting raw materials for synthesis biphasic calcium phosphate (BCP) and magnesium-doped biphasic calcium phosphate (Mg-BCP) powders

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ca/P molar ratio</th>
<th>Mg/Ca molar ratio</th>
<th>Ca+Mg/P molar ratio</th>
<th>Molarity (mol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ca</td>
</tr>
<tr>
<td>BCP</td>
<td>1.60</td>
<td>_</td>
<td>_</td>
<td>2.000</td>
</tr>
<tr>
<td>Mg-BCP</td>
<td>_</td>
<td>0.05</td>
<td>1.60</td>
<td>2.000</td>
</tr>
</tbody>
</table>

Based on the molar ratio in Table 3.2, the amount of the starting raw materials used for synthesis BCP and Mg-BCP powders in this research work can be determine in Table 3.3 and Table 3.4 respectively.

Table 3.3: The amount of the starting raw materials for synthesis biphasic calcium phosphate (BCP) powder

<table>
<thead>
<tr>
<th>Chemical formula</th>
<th>Solvent</th>
<th>Molarity (mol/ml)</th>
<th>Volume of solvent (ml)</th>
<th>Mole (mol)</th>
<th>Molecular Weight (g/mol)</th>
<th>Mass of raw material (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca(NO$_3$)$_2$.4H$_2$O</td>
<td>Distilled water</td>
<td>2.000</td>
<td>70.00</td>
<td>0.1400</td>
<td>236.15</td>
<td>33.0610</td>
</tr>
<tr>
<td>(NH$_4$)$_2$HPO$_4$</td>
<td>Distilled water</td>
<td>1.250</td>
<td>70.00</td>
<td>0.0875</td>
<td>132.05</td>
<td>11.5544</td>
</tr>
</tbody>
</table>
Table 3.4: The amount of the starting raw materials for synthesis magnesium-doped biphasic calcium phosphate (Mg-BCP) powder

<table>
<thead>
<tr>
<th>Chemical formula</th>
<th>Solvent (mol/ml)</th>
<th>Molarity</th>
<th>Volume of solvent (ml)</th>
<th>Mole (mol)</th>
<th>Molecular Weight (g/mol)</th>
<th>Mass of raw material (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca(NO₃)₂·4H₂O</td>
<td>Distilled water</td>
<td>2.000</td>
<td>70.00</td>
<td>0.14000</td>
<td>236.15</td>
<td>33.0610</td>
</tr>
<tr>
<td>Mg(NO₃)₂·6H₂O</td>
<td>Distilled water</td>
<td>0.100</td>
<td>70.00</td>
<td>0.00700</td>
<td>256.41</td>
<td>1.7949</td>
</tr>
<tr>
<td>(NH₄)₂HPO₄</td>
<td>Distilled water</td>
<td>1.313</td>
<td>70.00</td>
<td>0.09191</td>
<td>132.05</td>
<td>12.1367</td>
</tr>
</tbody>
</table>

3.2.2 Synthesis Powders of Biphasic Calcium Phosphate (BCP) and Magnesium-doped Biphasic Calcium Phosphate (Mg-BCP)

Aqueous precipitation method was used to synthesis powders of BCP and Mg-BCP in this research work.

Synthesized of BCP was done by first prepared source of calcium (Ca²⁺) precursor, Ca(NO₃)₂·4H₂O solution by dissolving in distilled water. Next, source of phosphate (PO₄³⁻) precursor, (NH₄)₂HPO₄ solution was added into source of calcium (Ca²⁺) precursor, Ca(NO₃)₂·4H₂O solution via titration technique and continuously stirred for 120 minutes. When (NH₄)₂HPO₄ solution added into Ca(NO₃)₂·4H₂O solution, precipitation take place immediately during the titration process. After the mixing process was done, the pH value of the solution was maintaining at pH 11 by dropping NH₄OH (25%) solution. After the completing mixing process, the solutions were stirred for another hour to ensure chemical reaction completely done before aging for approximately
24 hours. Aged solution was decanted and centrifuged to separate water and precipitation product.

For synthesis of Mg-BCP, the source of calcium (Ca^{2+}) precursor, Ca(NO_3)_2.4H_2O solution was added into source of magnesium (Mg^{2+}) precursor, Mg(NO_3)_2.6H_2O solution. The solution was stirred until both chemicals completely dissolved in this solution. Next, source of phosphate (PO_4^{3-}) precursor, (NH_4)_2HPO_4 solution was added into calcium-magnesium solution via titration technique and continuously stirred for 120 minutes. Precipitates were obtained in this solution because precipitation takes place immediately during the process. After the mixing process was done, 25% NH_4OH solution was added to maintain the pH of the mixture to 11. The solutions were stirred for another hour to ensure chemical reaction completely done before aging it for approximately 24 hours. Aged solution was decanted and centrifuged to separate water and precipitation product.

Afterward, the white precipitate produced need to be washed three times with 120 ml of water to remove any possible residues from the precipitate. The solution was filtered using Whatman 90 mm filtration set with 542 grade filter paper. The filter cake was dried in the oven at 90°C for 24 hours. The dried cake was crushed using agate mortar and pestle, then 90 µm particles were separated using a sieve.

Finally, the as-synthesized powders were then characterized using various technique such as Thermogravimetry (TG) and Differential Scanning Calorimetry (DSC), X-ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR) and Field Emission Scanning Electron Microscopy (FESEM). Figure 3.2 and Figure 3.3 show flowchart of the process for the synthesis of BCP and Mg-BCP powders via aqueous precipitation technique respectively.
3.2.3 Flowchart of Synthesis of Biphasic Calcium Phosphate (BCP) Powders

![Flowchart of Synthesis of Biphasic Calcium Phosphate (BCP) Powders](image)

Figure 3.2: Flowchart of the process for synthesis of biphasic calcium phosphate (BCP) powders
3.2.4 Flowchart of Synthesis of Magnesium-doped Biphasic Calcium Phosphate (Mg-BCP) Powders

Figure 3.3: Flowchart of the process for synthesis of magnesium-doped biphasic calcium phosphate (Mg-BCP) powders
3.3 Characterization for As-synthesized Biphasic Calcium Phosphate (BCP) and Magnesium-doped Biphasic Calcium Phosphate (Mg-BCP) Powders

Several methods were used to characterize the powders that had been synthesized. The BCP and Mg-BCP powders were characterized using TG-DSC, XRD, FTIR and FESEM.

3.3.1 Thermogravimetry (TG) and Differential Scanning Calorimetry (DSC)

TG will provide information on the change in the weight of the sample and enables the stoichiometry of a reaction to be followed directly. DSC will give in amount of the energy absorbed or released by sample. Simultaneous TG-DSC were performed using TGA/DSC 1 STAre System, Mettler Toledo that enable both quantitatively and qualitatively measurement to be done subjected to temperature program.

Test specimen must be dried in the oven about 100 °C. Then, it was cooled to room temperature and kept in desiccator. After that, test specimen and α-Al$_2$O$_3$ crucible were weighed accurately at 100-200 mg and 150-200 mg, respectively. α-Al$_2$O$_3$ crucible was used as reference material. Then, both were placed into two separate crucibles. Next, both crucibles with test specimen and reference material were transferred to sample carrier system. The measurement was set up at 10 °C/min of constant heat rate from 20 to 1100 °C in air atmosphere.

3.3.2 X-ray Diffraction (XRD)

In this research work, X-ray diffraction (XRD) was carried out by using a Bruker AXD D8 Advance. By using X-ray diffraction analysis, the degree of crystalline phase composition and crystallinity of powders that had been synthesized can easily be characterized. The diffractometer was operated at 40 kV and 110 mA with step size of 0.02° using a copper (Cu) target as radiation source which giving Kα radiation with a
wavelength, \( \lambda = 1.5406 \text{ Å} \). The scattered intensity was scanned in the 20 range which fixed between 10° to 70°. As a result, the data obtained is in the values for intensity and diffraction angle in the form of a graph. Then, by using X’pert HighScore Plus software, the diffraction pattern was analysed and matched with the standard reference data, International Centre of Standard Database (ICSD). Rietveld refinement and quantitative X-ray diffraction analysis were carried out on the collected patterns using the software, allowing the quantification of phases and identification of changes in unit cell parameters caused by the structural substitutions.

3.3.3 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR Analysis or FTIR Spectroscopy is an important technique in organic and inorganic chemistry that provides qualitative analysis of materials by producing an infrared absorption spectrum. FTIR always deal with the infrared region of the electromagnetic spectrum and the data were plotted either in transmittance or absorbance spectrum which allows the interpretation of the functional group present in the sample. In this research, by using FTIR Perkin Elmer Spectrum One, FTIR analysis was used to determine chemical constituents and chemical bonding that presence in BCP and Mg-BCP powders.

Earlier sample preparation stage involved grinding as-synthesized powders with addition of potassium bromide (KBr). The ratio of powders and potassium bromide is 1:9. These mixed powders must be grinded into fine powder form and must be homogenously. Afterwards, the mixed powders were pressed into a pellet by using a hydraulic hand press at 5 MPa and hold for 2 minutes. A transparent thin pellet was formed, and it was placed in the FTIR Perkin Elmer Spectrum One machine. The sample was characterized using transmittance mode at the wave number range was set up to be 4000 to 400 cm\(^{-1}\).
3.3.4 Field Emission Scanning Electron Microscopy (FESEM)

Surface morphologies of the as-synthesized BCP and Mg-BCP powders were characterized by FESEM.

In this experiment, FESEM (SUPRA 35VP ZEISS) was used to successfully capture the image of as-synthesized powders. However, since the as-synthesized powders were non-conductive materials, the preparation of sample must be done by including the coating process. This coating process which the as-synthesized powders was coated with thin layer of conductive material which is gold metal was done by using Polaron Division, SEM Coating System Machine. Typically, gold metal was used to coat the as-synthesized powders in order to avoid the accumulation of electrons on the surface of the sample. Then, the coated sample was put on the sample holder and placed into the chamber of FESEM machine.

Basically, the area to be examined was irradiated with finely focused electron beams in FESEM machine to generate different mode include secondary electrons (SE), back-scattered electrons (BSE), and characteristic X-rays. All these modes were provided different types of signals. Then, these signals were collected by detectors which formed a surface morphology on the selected area of sample and displayed on a cathode ray tube screen in three-dimensional images. The magnification of sample was captured using various magnifications.

3.4 Fabrication of Biphasic Calcium Phosphate (BCP) and Magnesium-doped Biphasic Calcium Phosphate (Mg-BCP) Pellets

3.4.1 Uniaxial Pressing

To produce pellets, the BCP and Mg-BCP powders were compacted using a hydraulic hand press machine (model Enerpac). Powders must be weight at about 0.5
gram then the weighted powders are placed in the 13 mm diameter die. To pelletize the powders, 50 MPa of uniaxial pressure was applied onto the die. The pressure was held continually around 120 seconds to ensure uniform pressure was applied to the samples. After that, the pressure was released slowly, and the pellet was pushed out from the die carefully.

### 3.4.2 Calcination of Green Body Biphasic Calcium Phosphate (BCP) and Magnesium-doped Biphasic Calcium Phosphate (Mg-BCP)

In this research work, calcination was conducted using chamber furnace (Lenton Muffle Furnace) to obtain pellets of BCP and Mg-BCP. This process is important because it will improve the mechanical properties of that product.

All pellets were placed in this furnace and calcined at different temperature of 600 °C, 700 °C and 800 °C in air atmosphere. The heating rate used was 5 °C/minutes, soaking time of 2 hours and subsequently cooling at the rate 5 °C/min as shown in Figure 3.4.

![Calcination profile of biphasic calcium phosphate (BCP) and magnesium-doped biphasic calcium phosphate (Mg-BCP) pellets](figure3.4.png)

**Figure 3.4:** Calcination profile of biphasic calcium phosphate (BCP) and magnesium-doped biphasic calcium phosphate (Mg-BCP) pellets
Table 3.5 shows the list of pellets and its calcination temperatures used in this research work.

Table 3.5: List of BCP and Mg-BCP pellets with increasing calcination temperatures and its codes

<table>
<thead>
<tr>
<th>Calcination Temperature (°C)</th>
<th>Sample</th>
<th>Sample Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>600</td>
<td>BCP</td>
<td>C6 BCP</td>
</tr>
<tr>
<td></td>
<td>Mg-BCP</td>
<td>C6 Mg-BCP</td>
</tr>
<tr>
<td>700</td>
<td>BCP</td>
<td>C7 BCP</td>
</tr>
<tr>
<td></td>
<td>Mg-BCP</td>
<td>C7 Mg-BCP</td>
</tr>
<tr>
<td>800</td>
<td>BCP</td>
<td>C8 BCP</td>
</tr>
<tr>
<td></td>
<td>Mg-BCP</td>
<td>C8 Mg-BCP</td>
</tr>
</tbody>
</table>
3.4.3 Flowchart of Fabrication of Biphasic Calcium Phosphate (BCP) and Magnesium-doped Biphasic Calcium Phosphate (Mg-BCP) Pellets

Figure 3.5: Flowchart of fabrication of biphasic calcium phosphate (BCP) and magnesium-doped biphasic calcium phosphate (Mg-BCP) pellets
### 3.5 Characterization for Biphasic Calcium Phosphate (BCP) and Magnesium-doped Biphasic Calcium Phosphate (Mg-BCP) Pellets

Several characterization techniques were used to evaluate calcined pellets. The calcined BCP and Mg-BCP pellets were characterized using X-ray diffraction (XRD) and Scanning Electron Microscopy (SEM). Also, several other characterizations are used such as linear shrinkage measurement, porosity and density measurement, and hardness test.

#### 3.5.1 X-ray Diffraction (XRD)

In this section, the XRD of calcined pellets were analysed with the same concepts and principles of XRD in Section 3.3.2. Rietveld refinement and quantitative X-ray diffraction analysis also were carried out on the collected patterns using the X’pert HighScore Plus software, allowing the quantification of phases and identification of changes in unit cell parameters caused by the structural substitutions.

#### 3.5.2 Volume Shrinkage Measurement

Volume shrinkage measurement was measured by calculated the change in the dimensions of the pellets before and after calcination process. Generally, the thickness and diameter of each pellet were measured three times by using digital Vernier calliper before and after calcination process. Then, by using the average thickness and diameter measurement obtained, the percentage of volume shrinkage was calculated by using Equation 3.1.
\[
\% V_s = \left( \frac{V_o - V_f}{V_o} \right) \times 100 \quad \ldots \text{(Equation 3.1)}
\]

where,

\begin{align*}
\% V_s & = \text{Percentage shrinkage in volume} \\
V_o & = \text{Volume before} \\
V_f & = \text{Volume after}
\end{align*}

3.5.3 **Bulk Density Measurement of Pellets**

In this research work, bulk density measurement of pellets can be measure based on Archimedes principle.

The pellets were assumed have open and closed pores. The pellets have been weighed before immersed in water (D). After that, the pellets were soaked in a beaker filled with water, and then placed in a vacuum desiccator. The vacuum was turned on for 2 hours so that air can be sucked out from the pellets pores and allowed the water to enter into it. Suspended weight (S) is a weight of the pellet that taken while the pellet was submerged in the water. Saturated weight (W) is weight of the pellet after immersed in the water, while open pores now filled with water. Both of weight, suspended weight and saturated weight, were measured using Sartorius balance model and bulk density apparatus respectively. Figure 3.6 shown the density measurement using Archimedes method.
Using Equation 3.2, the bulk density of calcined pellets was calculated. Three pellets were measured for each composition of BCP and Mg-BCP and the average value was taken.

\[
\rho_{\text{bulk}} \text{ (g/cm}^3\text{)} = \frac{D}{W-S}
\]  

...(Equation 3.2)

where,

\[
D = \text{Dry mass (g)}
\]
\[
S = \text{Suspended mass (g)}
\]
\[
W = \text{Saturated mass (g)}
\]
3.5.4 Hardness Test

Hardness is a measure of a material’s resistance to localized plastic deformation. Hardness test depend on measuring the degree of penetration of a material as indication of hardness.

The hardness of the calcined pellets was measured by utilizing Vickers Hardness Tester Mitutoyo HV-114. Vickers utilizes a diamond pyramid indenter. Before the test can be performed, specimen surface preparation such as grinding, and polishing are important to guarantee a well-defined indentation to be precisely measured.

Firstly, the pellets were ground utilizing emery papers according to the grit from the coarser to the finer one (800, 1000, 1200, 1500 and 2000). During grinding, the pellets were rotated 90° to ensure uniform grinding and the pellets were then clean by ultrasonic for 10 minutes before continuing to the following grit of the emery paper. Next, the pellets were polished using Impotech 10V Grinder Polisher. Alumina powders with size of 1.0 µm, 0.3 µm and 0.05 µm, respectively were utilized as the polishing media. The pellets were polished utilizing alumina powders as indicated by the size from the coarser to the finer one. After the pellets were polished by using 1.0 µm alumina, the pellets were cleaned by ultrasonic for 10 minutes, before polishing with next size of alumina. Grinding and polishing are critical as to stay away from any contamination as well as scratches on the pellets. The load applied to the pellets was settled to 1 kgf for 20 seconds. The summary of the Vickers microhardness measurement is schematically demonstrated in Figure 3.7.
After the load was applied, the resulting impression was observed under a microscope and the value of diagonal indent length was measured. This measurement was in hardness number (HV) as shown in Equation 3.3. Five measurements were performed for every pellets and the average values were taken.

\[
\text{Hardness (HV)} = \frac{1.854P}{\left(\frac{d_1+d_2}{2}\right)^2} \quad \text{...(Equation 3.3)}
\]

Where P is the applied load, d₁ and d₂ are the diagonal indent length in millimetre (mm) as indicated in Figure 3.7.
3.5.4.1 Field Emission Scanning Electron Microscopy (FESEM)

Surface morphologies and microstructural characterization of Mg-BCP pellets were inspected by FESEM.

In this experiment, since the pellets were non-conductive materials, the preparation of pellet must be done by including the coating process. The gold coating process was done by using Polaron Division, SEM Coating System Machine. Then, the coated pellet was put on the sample holder and placed into the chamber of FESEM machine.

Basically, the surface morphology on the selected area of pellet was displayed on a cathode ray tube screen in three-dimensional images. The pellet was captured using various magnifications.
CHAPTER 4
RESULTS AND DISCUSSIONS

4.1 Introduction

In this chapter, the results obtained from various experiments and characterizations conducted in this research work will be discussed. There were 4 main stages embarked in this research work. The first of this chapter presented the observation result get from synthesis process of BCP and Mg-BCP powders. This will be followed by the results and discussion on various characterization of as-synthesis BCP and Mg-BCP powders which were using aqueous precipitation method for synthesis. The third section represents the observation result of calcined BCP and Mg-BCP pellets. Finally, the fourth section represents the results and discussion on characterizations to the effect of calcination on Mg-BCP pellet properties.

4.2 Synthesis of Biphasic Calcium Phosphate (BCP) and Magnesium-doped Biphasic Calcium Phosphate (Mg-BCP) Powders

4.2.1 Observation

In this research work, the observation was done during synthesis process of BCP and Mg-BCP powders.

From observation, during titration of \((\text{NH}_4)_2\text{HPO}_4\) solution into \(\text{Ca(NO}_3)_2\cdot4\text{H}_2\text{O}\) solution, the \(\text{Ca(NO}_3)_2\cdot4\text{H}_2\text{O}\) solution transform from transparent to milky white solution because precipitation was occurred immediately. Figure 4.1 shows the milky white precipitate formed in this research work.
4.3 Characterization for As-synthesized Biphasic Calcium Phosphate (BCP) and Magnesium-doped Biphasic Calcium Phosphate (Mg-BCP) Powders

The as-synthesized BCP and Mg-BCP powders were then characterized using different techniques such as TG-DSC, XRD, FTIR and SEM analysis. XRD and FTIR analyses were used to confirm the phase formation of the as-synthesized BCP and Mg-BCP powders. Lastly, the morphology of the as-synthesized BCP and Mg-BCP powders were observed by FESEM. As-synthesis BCP powder was also used as standard reference for as-synthesized Mg-BCP powder during analysis.

4.3.1 TG-DSC Analysis

In this research work, thermal behaviour of as-synthesized BCP and Mg-BCP powders were determined by analysing with TG-DSC. TG-DSC provides the information on weight loss and heat energy change of as-synthesized BCP and Mg-BCP powders as
the function of temperature. Besides that, TG-DSC also gives information about the thermal event that occurs.

4.3.1.1 TG-DSC Analysis for As-synthesized Biphasic Calcium Phosphate (BCP)

The TG-DSC thermograms of as-synthesized BCP powder is shown in Figure 4.2. From the Figure 4.2, it indicates the existence of three temperature zones. At first and second zones which are in range from 50 °C to 300 °C, the weight loss were ~11 % and ~26 %, respectively. At this stage, it is indicating to the loss of absorbed water and lattice water. The removal of water process starts to occur at the first stage of mass loss. All the residual water was eliminated at this stage as the temperature increase without any effect on lattice parameters. At the second stage of mass loss, water in pores and inter-crystallite position requires higher temperature for release. This was because capillary effects stabilized the water. Moreover, the second stage of mass loss also indicating to the energy for restructuring or rearrangement of the structure when heated after loss of water. Corresponding DSC data revealed an endothermic reaction for as-synthesized Mg-BCP powder peaking at that range of temperature (Kim et al., 2012).

At the third zone which in range from 350 °C-450 °C, the weight loss was about ~7 %. At this stage, broad and low endothermic reaction was occurred. When as-synthesized BCP powder treated with heat until this temperature, it undergoes a reaction where monetite is decomposed to pyrophosphate and structural water is released from the material (Duncan et al., 2014). The decomposition event can be shown in Equation 4.1.

\[
2\text{CaHPO}_4 \rightarrow \gamma\text{-Ca}_2\text{P}_2\text{O}_7 + \text{H}_2\text{O} \quad \cdots \text{(Equation 4.1)}
\]
Figure 4.2: TGA-DSC curves for as-synthesized BCP powder

The summary of the TG-DSC curves for as-synthesized BCP powder is shown in Table 4.1.

Table 4.1: Summary of TG-DSC curves for as-synthesized BCP powder

<table>
<thead>
<tr>
<th>Technique</th>
<th>Characteristic of event</th>
<th>Decomposition zone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>TG</td>
<td>Initial temperature (°C)</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Final temperature (°C)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Mass variation (%)</td>
<td>~11</td>
</tr>
<tr>
<td>DSC</td>
<td>Characteristic of the peak</td>
<td>Endothermic</td>
</tr>
</tbody>
</table>
4.3.1.2 TG-DSC Analysis for As-synthesized Magnesium-doped Biphasic Calcium Phosphate (Mg-BCP)

The TG-DSC thermograms of as-synthesized Mg-BCP powder is shown in Figure 4.3. It indicates the existence of three temperature zone in the Figure 4.3. At first zone which is in range from 50 °C to 100 °C, the weight loss was ~5 %. Corresponding DSC data revealed an endothermic reaction for as-synthesized Mg-BCP powder peaking at that range of temperature. While, at the second zone which in range from 150 °C-250 °C, broad and low endothermic reaction was occurred with the weight loss was ~14 %. At the first stage of mass loss, the drying process starts to occur. All the residual water was eliminated at this stage as the temperature increase without bioceramic powder structure change. As the temperature increase, second stage of mass loss happened which indicating to the energy for restructuring or rearrangement of the structure after loss of water when heated. The loss of water at this stage indicating to the lattice water which requires higher temperature for release. This was because water in pores and intercrystallite locations is stabilized by capillary effects. Similar findings have been observed in other studies (Kim et al., 2012).

At the section zone which is in range from 650 °C-690 °C, the weight loss was about less than 1%. At this stage, another endothermic reaction was occurred. This endothermic reaction can be related with the decomposition of nonstoichiometric Mg-HA to whitlockite above 600 °C. Transformation of HA to β-TCP (whitlockite) characterised by exothermic reaction and followed by endothermic reaction. Combes et al. (2016) has reported that the exothermic effect is attributed to particle rearrangement of low-crystallinity phases. Also, the Mg contents in HA reduces the decomposition temperature from high temperature (~800 °C) to this range of temperature to form a magnesium substituted β-TCP or (Ca,Mg)₃(PO₄)₂. Thus, this indicated that substitution of Mg²⁺ can
stabilised the β-TCP phase. This is in agreement with similar finding reported by other researchers (Sopyan & Rahim, 2012).

![Figure 4.3: TGA-DSC curve for as-synthesized Mg-BCP powder](image)

The summary of the TG-DSC curve for as-synthesized BCP powder is shown in Table 4.2.

**Table 4.2: Summary of TG-DSC curve for as-synthesized Mg-BCP powder**

<table>
<thead>
<tr>
<th>Technique</th>
<th>Characteristic of event</th>
<th>Decomposition zone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>TG</td>
<td>Initial temperature (°C)</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Final temperature (°C)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Mass variation (%)</td>
<td>~5</td>
</tr>
<tr>
<td>DSC</td>
<td>Characteristic of the peak</td>
<td>Endothermic</td>
</tr>
</tbody>
</table>
4.3.2 XRD Analysis

In this research work, degree of crystalline phase composition and crystallinity of as-synthesized BCP and Mg-BCP powders were determined by analysing with XRD. Besides that, XRD provides the data of diffraction pattern in the values for intensity and diffraction angle. Then, the diffraction pattern was analysed and matched with the standard reference data, International Centre of Standard Database (ICSD).

4.3.2.1 XRD Analysis for As-synthesized Biphasic Calcium Phosphate (BCP)

The X-ray diffraction (XRD) pattern of the as-synthesized BCP powders produced by an aqueous precipitation method is shown in Figure 4.4. Based on XRD analysis, it is observed that the XRD pattern obtained comply with the standard XRD reference pattern for monetite and HA that reported in ICSD with is file number 98-000-5558 and 98-010-2187 respectively. Both phases composition present in the as-synthesized BCP powder also be supported by using Rietveld refinement analysis.

In this research work, monetite and HA standard patterns were used as a reference patterns for comparison with the pattern of as-synthesized BCP powder. Generally the main peaks of reference HA which are at $2\theta = 10.48^\circ$ (010), $2\theta = 24.91^\circ$ (012), $2\theta = 25.49^\circ$ (002), $2\theta = 26.01^\circ$ (012), $2\theta = 28.09^\circ$ (120), $2\theta = 29.57^\circ$ (112), $2\theta = 32.36^\circ$ (030), $2\theta = 33.59^\circ$ (022), $2\theta = 39.19^\circ$ (130), $2\theta = 45.73^\circ$ (041), $2\theta = 46.07^\circ$ (222), $2\theta = 48.90^\circ$ (123), $2\theta = 49.78^\circ$ (231), and $2\theta = 50.54^\circ$ (140) complied with the peaks of calcined BCP pellets.
From the XRD pattern of as-synthesized BCP powder, it is shown that the broad diffraction pattern and this is attributed to small crystallite size and poor crystallinity of sample forms. This is also supported by the crystallite size calculated from the X’pert HighScore Plus software in Table 4.3.

Table 4.3: Data obtained by XRD analysis

<table>
<thead>
<tr>
<th>Sample</th>
<th>HA Phase</th>
<th>Intensity (counts) (I_{121})</th>
<th>Position (°2θ) (I_{121})</th>
<th>d-spacing (Å) (I_{121})</th>
<th>a=b (Å)</th>
<th>c (Å)</th>
<th>Crystallite Size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As-synthesized BCP</td>
<td></td>
<td>253.08</td>
<td>31.6079</td>
<td>2.82217</td>
<td>9.45988</td>
<td>6.86047</td>
<td>78.0985</td>
</tr>
</tbody>
</table>
As mentioned earlier, the Rietveld refinement technique was used to determine the wt % of phase composition present in the sample. Table 4.4 reports the determined wt % of the phase composition for as-synthesized BCP powder and it is indicated only the formation of monetite and HA mixtures, with no other extra phases identified.

Table 4.4: Determined Rietveld quantification of as-synthesized BCP powder

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight percent of composition determined by Rietveld quantification (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As-synthesized BCP</td>
<td></td>
</tr>
<tr>
<td>Monetite</td>
<td>7.2</td>
</tr>
<tr>
<td>HA</td>
<td>92.8</td>
</tr>
<tr>
<td>β-TCP</td>
<td>0</td>
</tr>
</tbody>
</table>

4.3.2.2 XRD Analysis for As-synthesized Magnesium-doped Biphasic Calcium Phosphate (Mg-BCP)

Figure 4.5 demonstrated the XRD pattern of as-synthesized BCP and Mg-BCP powder which consisting fixed composition of Mg substituted into the BCP lattice. The XRD diffraction pattern of as-synthesized Mg-BCP powder was matched with standard HA diffraction patterns with ISCD file number of 98-010-2187. The phases composition present in the as-synthesized BCP powder also be supported by using Rietveld refinement analysis.

The main peaks which are at $2\theta = 26.46^\circ$ (021), $2\theta = 33.25^\circ$ (022), $2\theta = 33.32^\circ$ (112), $2\theta = 33.92^\circ$ (030), $2\theta = 35.17^\circ$ (022), $2\theta = 40.26^\circ$ (122), $2\theta = 47.33^\circ$ (041), $2\theta = 47.69^\circ$ (222), $2\theta = 49.06^\circ$ (132), $2\theta = 50.54^\circ$ (123), $2\theta = 51.39^\circ$ (231), $2\theta = 52.15^\circ$ (140), $2\theta = 53.02^\circ$ (042), $2\theta = 53.09^\circ$ (033), $2\theta = 53.97^\circ$ (141) and $2\theta = 54.34^\circ$ (004) based on Mg-BCP powder comply with major peak of HA.
Figure 4.5: XRD pattern of as-synthesized (a) Mg-BCP and (b) BCP powder that matched with main peaks of reference HA.

Table 4.5 reports the determined wt % of the phase composition for as-synthesized Mg-BCP powder. It is indicated only the formation of HA phase, with no other extra phases identified.

Table 4.5: Determined Rietveld quantification of as-synthesized Mg-BCP powder

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight percent of composition determined by Rietveld quantification (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monetite</td>
</tr>
<tr>
<td>As-synthesized Mg-BCP</td>
<td>0</td>
</tr>
</tbody>
</table>

From the XRD pattern of as-synthesized Mg-BCP powder, it is shown that the peaks were shifted slightly to the right. Based on Table 4.6, it was proven that the peaks were shifted slightly to the right as the main peak, which corresponds to the plane (1 2 1) increasing compare to the position of based as-synthesized BCP powder, which position...
at 31.6792. This might indicate the substitution of \( \text{Mg}^{2+} \) into the BCP structure. It is conveyed that doping of metal ion has an impact on the crystal configuration of HA. This occurs as the size mismatch between doped ion and calcium ion. Replacement of bigger ionic radius of \( \text{Ca}^{2+} \) (0.099 nm) with smaller ionic radius of \( \text{Mg}^{2+} \) (0.071 nm) can cause contraction and change in d-spacing as can be seen in Table 4.6. The narrowing of the crystal lattice of HA lead to reduction in the crystallinity. Thus, this explained the reason broadening of the pattern for as-synthesized Mg-BCP powder, as compared with as-synthesized BCP powder. There were also decrease in the intensity of the peaks as can be seen for as-synthesized Mg-BCP which commonly related with the decreasing in the crystallinity of HA.

Table 4.6: XRD data of as-synthesized BCP and Mg-BCP powders

<table>
<thead>
<tr>
<th>Sample</th>
<th>HA Phase</th>
<th>Intensity (counts ((I_{121})))</th>
<th>Position ( {^\circ 2\theta} ) ((I_{121}))</th>
<th>d-spacing ( \text{Å} ) ((I_{121}))</th>
<th>( a=b ) ( \text{Å} )</th>
<th>( c ) ( \text{Å} )</th>
<th>Crystallite Size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As-synthesized BCP</td>
<td></td>
<td>253.08</td>
<td>31.6079</td>
<td>2.82217</td>
<td>9.45988</td>
<td>6.86047</td>
<td>78.0985</td>
</tr>
<tr>
<td>As-synthesized Mg-BCP</td>
<td></td>
<td>125.46</td>
<td>31.8517</td>
<td>2.81529</td>
<td>9.45456</td>
<td>6.86286</td>
<td>92.1318</td>
</tr>
</tbody>
</table>

4.3.3 FTIR Analysis

FTIR analysis was used to identify chemical bonds in a molecule by producing an infrared absorption spectrum. Figure 4.6 shows the FTIR pattern of as-synthesized BCP
and Mg-BCP powders with the bands which are labelled accordingly for different functional groups. FTIR analysis of as-synthesized BCP and Mg-BCP powders confirmed the presence of the typical characteristic bands of different functional groups corresponding to the standard BCP. Based on FTIR analysis, the as-synthesized Mg-BCP powder showed the presence characteristic peaks of different functional groups that corresponding to the as-synthesized BCP was used as a reference for comparison.

![FTIR spectra of as-synthesized (a) BCP and (b) Mg-BCP powders](image)

Figure 4.6: FTIR spectra of as-synthesized (a) BCP and (b) Mg-BCP powders

As-synthesized BCP powder showed the main characteristic bands at 475, 574, 609, 966 and 1020-1120 cm\(^{-1}\), which referred to the vibrational modes of PO\(_4\) groups. The presence OH groups at 3570 cm\(^{-1}\) and 630 cm\(^{-1}\) have also been witnessed in the infrared spectra for as-synthesized powders which both bands represent stretching mode and bending mode respectively. FT-IR patterns show that the intensity of peak resolution of PO\(_4\) and OH bands are viewed with less intensity with the substitution of Mg\(^{2+}\). The presence of adsorbed water could also be detected from FT-IR spectra in the region around 3300–3600 cm\(^{-1}\) which usually retained during the aqueous precipitation process.
due to poorly crystalline apatite phase of as-synthesized powder. Other information from the FT-IR spectra of the powders is the presence of carbonates (CO$_3^-$) groups at 1660 cm$^{-1}$, which are due to the adsorption of species remaining from the aqueous precipitation. The presence of nitrates (NO$_3^-$) in the as-synthesized powders is clearly witnessed in the FTIR patterns in the region around at 1385 cm$^{-1}$ and the band at 875 cm$^{-1}$ resulting from the residual species in the as-synthesized powders tend to agree with the previous study (Jang et al., 2015).

4.3.4 FESEM Analysis

Surface morphologies and microstructural characterization of the as-synthesized BCP and Mg-BCP powders were inspected by using FESEM. Three magnifications were used in this research work which are 500 X, 2000 X and 5000 X.

Figure 4.7 and Figure 4.8 present FESEM micrographs of the as-synthesized BCP and Mg-BCP powders, respectively at three different magnification which are 500 X, 2000 X and 5000 X. From the SEM observation in both figure, the agglomeration of powder formed. Due to the agglomeration, it was rather difficult to estimate the actual particle size of the individual particles. At this state of study, it is assuming that the powders are in nano-size range. This is because when the particles are in nanometer size, Van der Waals force attraction between the nanoparticles most possibly leads to this formation of fine agglomerates (Mohammad et al. 2016). In order to get a better dispersed particle, dispersant could be added to prevent powders from aggregating. However, in the present stage of work, this was not emphasized.

A higher magnification micrograph in Figure 4.7 (c) highlights that there is a typical monetite morphology exists inside the structure of as-synthesized BCP powder. It can clearly be observed the formation of rectangular, plate-like crystal for monetite
morphology (Duncan et al., 2014). However, in Figure 4.8, the monetite morphology does not exist inside the structure of as-synthesized Mg-BCP powder. Based on the result obtained from XRD of as-synthesized BCP powder, low wt % of monetite phase can be detected. Thus, the finding from the FESEM micrographs of the as-synthesized BCP powder is supported with the result obtained from the XRD analysis.
Figure 4.7: SEM micrographs of the as-synthesized BCP powder at magnification of (a) 500 X, (b) 2000 X and (c) 5000 X

Rectangular, plate-like crystal
Figure 4.8: SEM micrographs of the as-synthesized Mg-BCP powder at magnification of (a) 500 X, (b) 2000 X and (c) 5000 X
4.4 Fabrication of Biphasic Calcium Phosphate (BCP) and Magnesium-doped Biphasic Calcium Phosphate (Mg-BCP) Pellets

4.4.1 Observation

In this research work, the observation was done during fabrication of BCP and Mg-BCP pellets.

From observation, white pellets of BCP and Mg-BCP formed before the calcination process. Additionally, no change in colour of those pellets after the calcination process. However, slightly change in dimension of the pellets happened after the calcination process. Figure 4.9 shows the white pellets formed before and after calcination process.

Figure 4.9: White pellets formed (a) before and (b) after calcination process
4.5 Characterization for Biphasic Calcium Phosphate (BCP) and Magnesium-doped Biphasic Calcium Phosphate (Mg-BCP) Pellets

4.5.1 XRD Analysis

The calcined BCP and Mg-BCP pellets were analysed by XRD analysis to check the phase formation and purity. Furthermore, as-synthesized BCP powder was used as reference for calcined BCP pellets which then was used as reference for Mg-BCP pellets.

4.5.1.1 XRD Analysis Comparison Between As-synthesized BCP and Calcined BCP Pellets

Figure 4.10 presents the XRD patterns of (a) as-synthesized BCP powder and BCP pellets calcined at (b) 600 °C, (c) 700 °C and (d) 800 °C. Analysis of the data obtain showed that all calcined BCP pellets were match with the standard XRD reference pattern of HA that reported in ISCD with file number 98-010-2187. Moreover, as-synthesized BCP was used as reference for calcined BCP pellets.

Calcined BCP pellets also showed a sharper and narrower XRD patterns, which can be translated as a crystalline structure was formed when the pellets undergo calcination process. By comparing the XRD patterns in Figure 4.10, as-synthesized BCP powders before calcination showed a broad peak as compared to the BCP pellets after calcination. This trend was also observed by (Kumar et al., 2012).
Figure 4.10: XRD patterns of (a) as-synthesized BCP powder and BCP pellets calcined at (b) 600 °C, (c) 700 °C and (d) 800 °C

Table 4.7 reports the determined wt % of the phase composition for as-synthesized BCP powder, C6 BCP, C7 BCP and C8 BCP. It is indicated only the formation of HA phase, with no other extra phases identified. It can be concluded that calcination process was done without changing the composition of BCP as it is confirmed that single phase of HA was formed.
Table 4.7: Determined Rietveld quantification of as-synthesized BCP powder and BCP pellets with different calcination temperature

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight percent of composition determined by Rietveld quantification (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monetite</td>
</tr>
<tr>
<td>As-synthesized BCP</td>
<td>0</td>
</tr>
<tr>
<td>C6 BCP</td>
<td>0</td>
</tr>
<tr>
<td>C7 BCP</td>
<td>0</td>
</tr>
<tr>
<td>C8 BCP</td>
<td>0</td>
</tr>
</tbody>
</table>

Other than the XRD patterns become sharper, there were also changes in lattice parameters and crystallite size. These changes can be observed from XRD data based on Table 4.8. There was slightly expansion on a-axis, also on c-axis after calcination. Due to calcination process, the crystallite size constantly increases as it is being able to growth with decreasing in term of micro-strain. The increase of the crystallite size of BCP directly resulted in the increased stability, respectively (Guo et al., 2003).

Table 4.8: Data obtained by XRD analysis for as-synthesized BCP, C6 BCP, C7 BCP and C8 BCP

<table>
<thead>
<tr>
<th>Sample</th>
<th>Intensity (counts) (I_{121})</th>
<th>Position (°2θ) (I_{121})</th>
<th>d-spacing (Å) (I_{121})</th>
<th>a=b (Å)</th>
<th>c (Å)</th>
<th>Crystallite Size (nm)</th>
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<tbody>
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<td>9.45988</td>
<td>6.86047</td>
<td>78.0985</td>
</tr>
<tr>
<td>C6 BCP</td>
<td>150.03</td>
<td>32.2077</td>
<td>2.77706</td>
<td>9.46276</td>
<td>6.89844</td>
<td>125.1</td>
</tr>
<tr>
<td>C7 BCP</td>
<td>125.57</td>
<td>32.3852</td>
<td>2.76224</td>
<td>9.46929</td>
<td>6.90370</td>
<td>142.7</td>
</tr>
<tr>
<td>C8 BCP</td>
<td>155.06</td>
<td>31.8138</td>
<td>2.81054</td>
<td>9.47791</td>
<td>6.90250</td>
<td>792.9</td>
</tr>
</tbody>
</table>
4.5.1.2 XRD Analysis Comparison Between Calcined BCP Pellets and Calcined Mg-BCP Pellets

Figure 4.11 shows the XRD patterns for BCP and Mg-BCP pellets calcined at 600 °C, 700 °C and 800 °C. In this analysis, calcined BCP pellets were used as reference for calcined Mg-BCP pellets. Based on Figure 4.11, additional peaks that obtained which indicate to formation of tricalcium phosphate, in this case, β-TCP can be detected after the calcination process of Mg-BCP. It is observed that the XRD patterns of calcined Mg-BCP pellets obtained comply with the standard XRD reference pattern for HA and whitlockite (β-TCP) that reported in ISCD with file number 98-010-2187 and 98-000-0800 respectively.

Figure 4.11: XRD patterns of BCP and Mg-BCP pellets that matched with main peaks of reference HA and β-TCP
The wt % of composition determined by Rietveld quantification for as-synthesized Mg-BCP and calcined Mg-BCP pellets are shown in Table 4.9. Based on Table 4.9, it clearly be observed that the formation of HA and β-TCP mixture in the structure of Mg-BCP pellet starts at the calcination temperature of 700 °C. Nevertheless, at calcination temperature of 600 °C, it is indicated only the formation of HA, with no other extra phases identified. Thus, the finding from the XRD patterns of the calcined Mg-BCP pellets is supported with the result obtained from the TG-DSC analysis of as-synthesized Mg-BCP powder which decomposition of nonstoichiometric HA to β-TCP started at 650 °C. The Mg²⁺ contents in HA reduces the decomposition temperature from high temperature (~800 °C) to this temperature to form a magnesium substituted β-TCP. This is in agreement with similar finding reported by other researchers.(Kannan et al., 2007)

Also, as the calcination temperature increase, the wt % of HA and β-TCP mixture will change for calcined Mg-BCP pellets. Based on Table 4.9, it can be shown that the formation of HA phase decrease while the formation of β-TCP increase in the structure of Mg-BCP pellet as the calcination temperature increase. Thus, the result tends to agree with the previous study (Kannan et al., 2007).

Table 4.9: Determined Rietveld quantification of calcined BCP and Mg-BCP pellets

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Weight percent of composition determined by Rietveld quantification (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monetite</td>
</tr>
<tr>
<td>C6 BCP</td>
<td>0</td>
</tr>
<tr>
<td>C6 Mg-BCP</td>
<td>0</td>
</tr>
<tr>
<td>C7 BCP</td>
<td>0</td>
</tr>
<tr>
<td>C7 Mg-BCP</td>
<td>0</td>
</tr>
<tr>
<td>C8 BCP</td>
<td>0</td>
</tr>
<tr>
<td>C8 Mg-BCP</td>
<td>0</td>
</tr>
</tbody>
</table>
From the XRD patterns of calcined BCP and Mg-BCP pellets, it was shown that the peaks were shifted slightly to the right for calcined Mg-BCP pellets. Evidence for this was provided in Table 4.10. It can be seen that the shifted peak corresponds to the plane (1 2 1) increasing when compared to the position of based calcined BCP pellets. The peak was related to substitution of Mg$^{2+}$ into BCP structure as a dopant which was affected the crystal configuration of HA due to mismatch in term of size between doped ion and calcium ion. The effect of replacement between bigger ionic radius of Ca$^{2+}$ (0.099 nm) with smaller ionic radius of Mg$^{2+}$ (0.071 nm) on crystal configuration because of contraction resulted changed in d-spacing. The narrowing of crystal lattice of HA lead to reduction in crystallinity. This was supported by decreasing in the intensity of peaks. Generally, the peaks intensity decreased with decreasing in crystallinity and crystal size of HA. It can be concluded that there was a reason broadening of pattern for calcined Mg-BCP pellets.

Table 4.10: Data obtained by XRD analysis for BCP and Mg-BCP pellets

<table>
<thead>
<tr>
<th>Sample</th>
<th>Intensity (counts) ($I_{121}$)</th>
<th>Position ($^\circ 2\theta$) ($I_{121}$)</th>
<th>d-spacing (Å) ($I_{121}$)</th>
<th>$a=b$ (Å)</th>
<th>$c$ (Å)</th>
<th>Crystallite Size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C6 BCP</td>
<td>150.03</td>
<td>32.2077</td>
<td>2.77706</td>
<td>9.46276</td>
<td>6.89844</td>
<td>125.1</td>
</tr>
<tr>
<td>C6 Mg-BCP</td>
<td>75.21</td>
<td>32.3918</td>
<td>2.76169</td>
<td>9.46751</td>
<td>6.86191</td>
<td>76.8</td>
</tr>
<tr>
<td>C7 BCP</td>
<td>125.57</td>
<td>32.3852</td>
<td>2.76224</td>
<td>9.46929</td>
<td>6.90370</td>
<td>142.7</td>
</tr>
<tr>
<td>C7 Mg-BCP</td>
<td>34.41</td>
<td>32.6566</td>
<td>2.73991</td>
<td>9.42791</td>
<td>6.87315</td>
<td>84.8</td>
</tr>
<tr>
<td>C8 BCP</td>
<td>155.06</td>
<td>31.8138</td>
<td>2.81054</td>
<td>9.47791</td>
<td>6.90250</td>
<td>792.9</td>
</tr>
<tr>
<td>C8 Mg-BCP</td>
<td>49.35</td>
<td>32.2104</td>
<td>2.77684</td>
<td>9.24230</td>
<td>6.96910</td>
<td>424.0</td>
</tr>
</tbody>
</table>
4.5.2 Volume Shrinkage Measurement Analysis

Volume shrinkage measurement was conducted to identify the shrinkage behaviour of BCP and Mg-BCP after being calcined. As the green body of pellets undergo calcination process, it decreases in volume and reduction of dimensions as ceramic grains being compacted and bond to each other (Ring & Ring, 1996).

Based on Figure 4.12, the percentage of shrinkage for volume of all calcined pellets have shown increasing after the calcination process. The trends of the percentage volume shrinkage shown uniformly for all six different calcined pellets. Percentage shrinkage in terms of volume (% Vs) approximately in a range 3.4-10.1 %.

As compared in term of BCP sample group, it is observed that C8 BCP has the highest percentage in shrinkage for volume and then followed by C7 BCP and C6 BCP. While, as compared in term of Mg-BCP sample group, it is observed that C8 Mg-BCP has the highest percentage in shrinkage for volume and then followed by C7 Mg-BCP and C6 Mg-BCP. Based on result from both sample group, it clearly shown that the calcination temperature will affect in the shrinkage of the pellets. As the calcination temperature increase, the percentage in volume shrinkage of pellets also become increase. By substitution of magnesium into the BCP structure, the percentage in volume shrinkage of pellets will decrease. This was due to HA to β-TCP phase transition as detected in TG-DSC curve of as-synthesized Mg-BCP in Figure 4.4 (Section 4.3.1.2). The difference of the size and shape of present phase occurred during phase transformation and it will inhibit the densification along with volume shrinkage (Xue et al., 2008; Champion, 2013).

For BCP group samples, shrinkage percentage in terms of volume in the range of 6.13-10.1%. On the other hand, for Mg-BCP group samples, shrinkage percentage in
terms of volume in a range 3.43-6.53%. When comparing between both group sample, BCP group samples stay among the highest in volume shrinkage when compared with Mg-BCP group samples. It is clearly shown that by substitution Mg$^{2+}$ into the BCP structure will affect in the shrinkage of the calcined pellets. By substitution of magnesium into the BCP structure, the percentage in volume shrinkage of pellets will decrease. This was happened because the lattice changes of apatite structure by substitution of Mg$^{2+}$ (Sader et al., 2009). Also, by substitution of Mg$^{2+}$ into the apatite structure will reduce the $\beta$-TCP phase transformation temperature. Thus, with the formation of $\beta$-TCP, it will inhibit the densification along with volume shrinkage (Xue et al., 2008; Champion, 2013).

![Graph showing percent volume shrinkage vs calcination temperature]

Figure 4.12: Shrinkage percentage in volume of BCP and Mg-BCP with increasing calcination temperature

4.5.3 Bulk Density Measurement Analysis

When undergo calcination process, the porosity of the pellets is removed and causes it to completely densify. As porosity removed, the pellets also encounter reduction in terms of its surface area. As surface area was reduce, there will be change in density
and stress is create. As the pellets undergo calcination process, pellets will decrease in volume and increase in density (Ring & Ring, 1996).

Thus, it can be said that the bulk density result of the pellets co-related with the result of shrinkage. Where, the highest density should experience highest shrinkage. In this experiment, the bulk density of all the calcined pellets were determined utilizing Archimedes principle. The bulk density of calcined pellets was measured using Equation 3.2 (Section 3.5.3).

Figure 4.14 shows the bulk density result of BCP and Mg-BCP pellets with increasing calcination temperature. Overall, it can be seen that, the effect calcination temperature for BCP and Mg-BCP pellets resulted in slightly decrease in bulk density for both pellets and never reaches the theoretical value for HA (3.16 g/cm³) and β-TCP (3.07 g/cm³). Therefore, it reveals the calcination temperature may affect the formation of porosity on the pellet (Eliaz & Metoki, 2017). The porosity formation on the pellet increase along with increasing calcination temperature. Consequently, the bulk density of pellet become decrease.

It is also noted that by substitution of Mg²⁺ into the BCP resulted in slightly increase in the range of 0.38-2 % for bulk density of pellets. This is due to inhibition of grain growth of BCP pellet (Eliaz & Metoki, 2017). Mg²⁺ incorporation or substitution on the surface of BCP will affect on the grain growth along with porosity formation on the pellet surface. Thus, this may affect the bulk density shows in Figure 4.13 which Mg-BCP pellets have higher bulk density compared to BCP at three different calcination temperatures (Ryu et al., 2004).
Figure 4.13: Bulk density results of BCP and Mg-BCP with increasing calcination temperature

4.5.4 Hardness Test Analysis

Figure 4.14 shows the hardness plots of BCP and Mg-BCP with increasing calcination temperature. Altogether, there obvious trend of the hardness value is seen which slightly decrease when the calcination temperature of BCP increase. However, for Mg-BCP, the hardness value was obviously decrease when the calcination temperature increase. This can be explained by the decreasing in bulk density measurement for both BCP and Mg-BCP pellets with increasing calcination temperature. As the calcination temperature increase, it induced the formation of porosity on the BCP and Mg-BCP pellets. Larger amount of porosity will reduce the hardness value at higher calcination temperature.

It is also noted that by substitution of Mg$^{2+}$ into the BCP pellets resulted in decrease in hardness value. The decrease in hardness value was attributed to phase transformation of HA to β-TCP. HA has mechanical strength which much higher when compared with β-TCP which it is accompanied by a sudden dilatometric expansion (Kannan et al., 2007; Champion, 2013). Thus, by substitution of Mg$^{2+}$ into the apatite
structure of BCP, it may induce the formation of β-TCP in the BCP structure and the hardness value will drop (Kannan et al., 2007). Mg$^{2+}$ also may affect the size and shape of current phases along with creation of microstrain that leads to lower hardness of BCP pellets.

![Figure 4.14: Hardness test results of BCP and Mg-BCP pellets with increasing calcination temperature](image)

**4.5.4.1 FESEM Analysis**

Surface morphologies and microstructural characterization of the Mg-BCP pellets were inspected by using FESEM. Mg-BCP pellets which calcined at 600 °C (C6 Mg-BCP) and 800 °C (C8 Mg-BCP) were inspected in this research work based on the highest and the lowest hardness value obtained. Three magnifications were used in this research work which are 5000 X, 10000 X and 30000 X.

Figure 4.15 shows FESEM micrographs C6 Mg-BCP and C8 Mg-BCP at magnification 5000 X, 10000 X and 30000 X. A higher magnification micrograph in Figure 4.15 highlights the surface porosity formation on the C6 Mg-BCP and C8 Mg-
BCP. The amount of porosity on C8 Mg-BCP was higher when compared with C6 Mg-BCP. The porosity increased with the calcination temperature and thus the finding from the FESEM micrographs is supported with the result obtained from the bulk density measurement analysis. When the calcination temperature increase, the bulk density will decrease along with increasing porosity formation in structure of Mg-BCP pellet. This may probably the secondary phase of β-TCP, were present on the surfaces of pellet (Ergun et al., 2002). This is agreed with the result of XRD for Mg-BCP pellets which β-TCP was detected at calcination temperature of 700 °C and 800 °C.
Figure 4.15: FESEM micrographs of C6 Mg-BCP (left) and C8 Mg-BCP (right) at magnification of (a) 5000 X, (b) 10000 X and (c) 30000 X
CHAPTER 5
CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In this research work, biphasic calcium phosphate (BCP) and magnesium-doped biphasic calcium phosphate (Mg-BCP) were synthesized using aqueous precipitation method at room temperature (RT). BCP powder was synthesized and be used as in this research work. XRD and FTIR analyses of the as-synthesized BCP powders showed that the powder produced monetite and HA phases. While, for as-synthesized Mg-BCP, it was confirmed that the composition of powder produced was HA and free of secondary phase. Morphology analysis of the as-synthesized BCP and Mg-BCP demonstrated that loosely aggregated fine particles. Additionally, rectangular, plate-like crystal can be seen from FESEM analysis of as-synthesized BCP.

Next, both as-synthesized BCP and Mg-BCP powders were used in the fabrication of pellets via uniaxial pressing method. Calcination was performed on BCP and Mg-BCP pellets at temperature of 600 °C, 700 °C and 800 °C in normal atmosphere. Based on XRD and FTIR analyses, it has confirmed that only the formation of HA phase detected in the all BCP pellets. While, secondary phase of β-TCP started to form in the Mg-BCP pellets which been calcined at 700 °C. As the calcination temperature increase to 800 °C, the amount of β-TCP phase increased (~5-22 wt%). All calcined pellets revealed sharper and narrower peaks as compared to their as-synthesized powders based on XRD analysis results.

The calcined BCP and Mg-BCP pellets were also tested for their percentage of volume shrinkage, bulk density measurement and Vickers hardness test. The percentage of volume shrinkage shows the increasing trend of data with increasing calcination
temperature where approximately in a range 6-10.2% for BCP pellets and approximately in a range 3.4-6.6% for Mg-BCP pellets. Moreover, the bulk density measurement shows the decreasing trend of data with increasing calcination temperature, where the data in range 1.455-1.522 g/cm³ for BCP pellets and the data in range 1.460-1.553 g/cm³ for Mg-BCP pellets. Furthermore, the hardness values of BCP and Mg-BCP pellets show the decreasing trend of data with increasing calcination temperature, where the data shown by BCP and Mg-BCP pellets were in range 48.8-52.2 HV and 13.2-43.8 HV respectively. Furthermore, based on the highest and the lowest result of hardness, the surface morphology of C6 Mg-BCP shows the lowest amount of porosity formation compared to C8 Mg-BCP. Thus, based on those results obtained in volume shrinkage, bulk density measurement and Vickers hardness test, it proved that the substitution of Mg²⁺ into the apatite structure will induce the formation of β-TCP which is naturally low in hardness value along with increasing calcination temperature. It will reduce the hardness value of BCP.

5.2 Recommendation for Future Work

The current research work required further improvement for future work, in order to improve the outcome of this research work. The following are a few suggestions that can be for future work:

1. To investigate different combination of ionic substitutions in the BCP structure as to closely mimic the bone mineral content.
2. To optimize calcination parameters such as calcination temperature, heating rate and soaking time in order to improve in densification of calcined product
3. Further investigation in term of biological properties, where conducting both in-vivo and in-vitro bioactivity, to strengthen the outcome of the research work.
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