INFLUENCE OF ACIDIC CALCIUM PHOSPHATE SOLUTION CONCENTRATION ON THE PROPERTIES OF DICALCIUM PHOSPHATE DIHYDRATE COATED ON β-TRICALCIUM PHOSPHATE PELLETS

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by

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DECLARATION

I hereby declare that I have conducted, completed the research work and written the dissertation entitled **"Influence of Acidic Calcium Phosphate Solution Concentration on The Properties of Dicalcium Phosphate Dihydrate Coated On β-Tricalcium Phosphate Pellets"**. I also declare that it has not been previously submitted for the award of any degree or diploma of similar title as this for any other examining body or university.

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Witness by

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF SYMBOLS

α	Alpha
β	Beta
θ	Theta
°	Degree
μ	Micron
ρ	Density
π	Pi
nm	Nanometer
wt%	Weight percent
°C/min	Degree celcius per minute
°C	Degree celcius
mmol/L	Millimoles per litre
cm^{-1}	Wavelength

LIST OF ABBREVIATIONS

α-TCP	α-TRicalcium Phosphate
AFM	Atomic Force Microscopy
A.u	Arbitary unit
ACP	Amorphous Calcium Phosphate
β-TCP	β-TRicalcium Phosphate
$CaCO_3$	Calcium Carbonate
CaP	Calcium Phosphate
Ca/P ratio	Calcium to Phosphate ratio
DCPD	Dicalcium Phosphate Dihydrate
DTS	Diametral Tensile Strength
FESEM	Field Emission Scanning Electron Microscope
FTIR	Fourier Transform Infrared Spectroscopy
H_3PO_4	Phosphoric Acid
HAp	Hydroxyapatite
HBSS	Hank's Balanced Salt Solution
ICDD	International Centre for Diffraction Data
ICP-OES	Inductive coupled plasma-optical emission spectrometry
L/P ratio	Liquid to pellets ratio
MCPM	Monocalcium Phosphate Monohydrate
MPa	Mega Pascal
OCP	Octacalcium Phosphate
TCP	Tricalcium Phosphate
XRD	X-ray Diffraction

PENGARUH KEPEKATAN LARUTAN KALSIUM FOSFAT BERASID TERHADAP SIFAT-SIFAT PELET B-TRIKALSIUM FOSFAT DISALUTI DIKALSIUM FOSFAT DIHIDRAT

ABSTRAK

Matlamat kajian ini adalah untuk mengkaji kesan kepekatan larutan kalsium fosfat berasid dalam penghasilan pelet β-trikalsium fosfat yang disaluti oleh dikalsium fosfat dihidrat (DCPD). Pelet β-TCP akan didedahkan kepada larutan kalsium fosfat berasid selama 1, 3 dan 5 jam bagi menghasilkan lapisan salutan DCPD di atas pelet β-TCP melalui tindak balas penguraian-pemendakan. Beberapa kaedah pencirian seperti Pembelauan Sinar-X (XRD), Spektroskopi Inframerah Transformasi Fourier (FTIR), Mikroskop Elektron Imbasan (SEM), Mikroskop Daya Atom (AFM), analisis keliangan dan ujian mampatan telah dijalankan ke atas spesimen. Analisa XRD dan SEM menunjukkan jumlah kristal DCPD yang termendak di atas pelet akan meningkat apabila kepekatan larutan kalsium fosfat berasid ditingkatkan. Peningkatan jumlah pembentukan DCPD akan mengurangkan keliangan sehingga 20% dan meningkatkan kekuatan mekanikal spesimen hingga mencapai 40%. Sementara itu, analisis XRD dan SEM menunjukkan bahawa pembentukkan hidroksiapatit (HAp) akan meningkat apabila masa rendaman bagi pelet β-TCP yang didedahkan selama 3 jam dengan menggunakan kepekatan larutan kalsium fosfat berasid yang berbeza dalam larutan garam seimbang Hank (HBSS) ditingkatkan pada keadaan fisiologi badan. Oleh itu, berdasarkan kajian ini menunjukkan bahawa jumlah penghasilan salutan DCPD di atas pelet β-TCP dapat dikawal dengan mengubah kepekatan larutan kalsium fosfat berasid yang dapat mencetuskan pembentukan HAp pada keadaan fisiologi badan.

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ABSTRACT

The aim of this study is to investigate the effect of acidic calcium phosphate solution concentration in fabricating dicalcium phosphate dihydrate (DCPD) coated on β-TCP pellets. β-TCP pellets were exposed to an acidic calcium phosphate solution for 1, 3 and 5 hours in order to obtain DCPD-coated layer on β-TCP pellets through dissolution-precipitation reaction. Several characterization methods such as X-Ray Diffraction (XRD), Fourier-Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscope (SEM), Atomic Force Microscope (AFM), porosity analysis and compression test were carried out on specimen. The analyses of XRD and SEM showed that the amount of DCPD crystal precipitated on the pellets were increased when increasing the acidic calcium phosphate solution concentration. Increasing the amount of DCPD formation will reduced the porosity until 20% and increased the mechanical strength of specimen up to 40%. Meanwhile, XRD and SEM analyses showed that hydroxyapatite (HAp) formation were increased after increasing the soaking time of β-TCP pellet treated for 3 hours using different concentration of acidic calcium phosphate solution in Hank's Balanced Salt Solution (HBSS). Therefore, based on this study shows that the amount of DCPD coated on β-TCP pellet could be regulated by changing the concentration of acidic calcium phosphate solution that induced HAp formation at physiological body condition.

CHAPTER 1

INTRODUCTION

1.1 Background of Research

Calcium phosphate (CaP) is one of the promising bone substitute material that had been widely used in clinics and hospitals. This material are well known for their good biocompatibility inside the body (Kwon et al., 2003). In addition, calcium phosphate also have the ability to regenerate new bone formation when being implanted in the body (Dong et al., 2002). Moreover, CaP had bioactive properties where it can induced specific biologic response with living tissues (Veis et al., 2009). Besides, CaP also having an important roles in human and animal daily lives due to the main components of the bone and teeth containing calcium phosphate itself (Habraken et al., 2016).

Currently, calcium phosphate bone substitute are being applied in various applications such as orthopaedic, dentistry and maxillofacial surgery. Also, CaP had widely studied as potential candidate for drug delivery application as they are compatible with the drugs (Cheng and Kuhn, 2007) such as gentamicin (Sundblom, Gallinetti, Birgersson, Engqvist, & Kihlström, 2019) and vancomycin (Uchida et al., 2018). Moreover, in the application of bone tissue engineering, CaP are mainly used as artificial bone substitute which help to induced formation of new bone (Canillas et al., 2017). Nowadays, calcium phosphate bone substitute materials has been produces in various shapes such as calcium phosphate cements (Xu et al., 2006), coatings (Paital and Dahotre, 2009), blocks (Lim et al., 2015), pellets (Baradari et al., 2012) and scaffolds (Shepherd and Best, 2011).

Beta-tricalcium phosphate (β-TCP) is one of the calcium phosphate that had been widely used due to their good biodegradability and biocompatibility despite higher dissolution after being implanted in the body. While dissolution process occurs in the body, osteoclast cell will resorbed the β-TCP and new bone formation will deposited on the β-TCP by osteoblast cells (Billington & Reid, 2019). Although, β-TCP show good new bone formation after implantation, its osteoconductivity are still inferior with the natural bone after implantation takes place in the large bone defect area. Therefore, recent study by Shariff et al., (2017) reported that osteoconductivity of β-TCP bone substitute in large bone defect size (above 6mm) was improved after coating β-TCP granular surfaces with a layer of dicalcium phosphate dihydrate (DCPD). Based on this idea, in this study, influence of acidic calcium phosphate solution concentration in fabricating bi-layer DCPD-coated β-TCP pellet will be investigated and its biological response towards Hank's Balanced salt Solution (HBSS) will be evaluated.

1.2 Problem Statements

Beta-tricalcium phosphate (β-TCP) had been widely used as artificial bone substitutes due to their similar chemical composition with natural bone. This advantages contributed to the good biocompatibility and osteoconductivity of β-TCP bone substitute. However, the new bone formation rate of β-TCP bone substitute is still inferior towards large bone defect size due to quick dissolution of β-TCP in large bone defect area (Zheng et al., 2014). Therefore, if new bone formation rate of β-TCP can be enhanced, its clinical applications could be enhanced.

Recent study by Shariff et al., (2017) had reported that the osteoconductivity of β-TCP were improved by coating β-TCP granular surfaces with a layer of dicalcium

phosphate dihydrate (DCPD). Histological evaluation using rats calvarial model shows that the β-TCP granules coated with 5 mass% amount of DCPD shows the largest new bone formation after implanted in rat calvarial for 2 and 4 weeks. Besides, in-vivo studies done by Fukuda et al., (2018) and Eddy et al., (2018) found that the presence of DCPD phase in β-TCP granular cement were significantly improved the new bone formation after implanted in 9 mm of rat calvarial bone defect size. Although this studies succeed to prove that DCPD phase can improved the new bone formation rate of β-TCP granular, there is no clearly study has been carried out in order to understand the correlation between in-vivo and in-vitro responses towards bi-layer DCPD-coated on β-TCP granular. Previous study had reported that the granular shape specimens show drawbacks during cell studies such as high probability of cells to be detached from the specimens due to the irregular shape of granules (Mebarki et al., 2017).

Meanwhile, there is a study reported that advantageous of pellet shape specimen for cell studies purposes. Study by Huang et al., (2017) found that cell can be easily attached on the pellet surface specimen during in-vitro study. This is due to the pellets shaped having flat shape surface while granules have irregular shape surface. Therefore, in this study, effect of acidic calcium phosphate concentration in fabricating bi-layer DCPD-coated on β-TCP pellet will be investigate and its biological activity will be tested using Hank's Balanced Salt Solution (HBSS).

1.3 Research Objectives

1. To investigate the effect of acidic calcium phosphate solution concentration in fabricating bi-layer DCPD-coated β-TCP pellet and its mechanical properties.

2. To evaluate hydroxyapatite formation on bi-layer DCPD-coated β-TCP pellet after exposing with Hank's Balanced Salt Solution at physiological body condition.

1.4 Research Approach

To determine the effect of acidic calcium phosphate solution in fabricating bi-layer DCPD-coated β-TCP pellet. There are two different concentration of acidic calcium phosphate solution were used which 50 mmol/L MCPM–25 mmol/L H_3PO_4 and 75mmol/L MCPM-25 mmol/L H_3PO_4. The exposure time for the coating process of DCPD on β-TCP pellet were set to at 1, 3 and 5 hours.

From the first stage, the characterization of bi-layer DCPD-coated β-TCP pellet were examined using X-ray Diffraction (XRD) which used to analyse the phase compositional present in the pellet. The presence of functional group in the specimen was analysed using Fourier Transform Infrared spectroscopy (FTIR). The surface and cross section morphology of the pellets were examined using Field Emission Scanning Electron Microscopy (FESEM). Moreover, Atomic Force Microscopy (AFM) was used to analyse the surface topography and surface roughness of the specimens. The relationship between porosity and compressive strength test was conducted in order to evaluate the mechanical properties of the pellets. Next, the pH analysis was carried out to determine the pH value of the HBSS solution after being exposed with the pellets.

Then, dissolution behaviour of Ca and P ions for the specimens were measured using Inductive coupled plasma-optical emission spectrometry (ICP-OES).

Based on the characterization in the first stage, specimen treated for 3 hours using both concentration of acidic calcium phosphate solution were selected for biological evaluation using HBSS solution at physiological body condition. Assessment of hydroxyapatite formation on the bi-layer DCPD-coated β-TCP pellet were evaluated using XRD, FTIR, FESEM and AFM. The method of producing bi-layer DCPD-coated β-TCP pellet and its biological test were summarized in Figure 1.1.

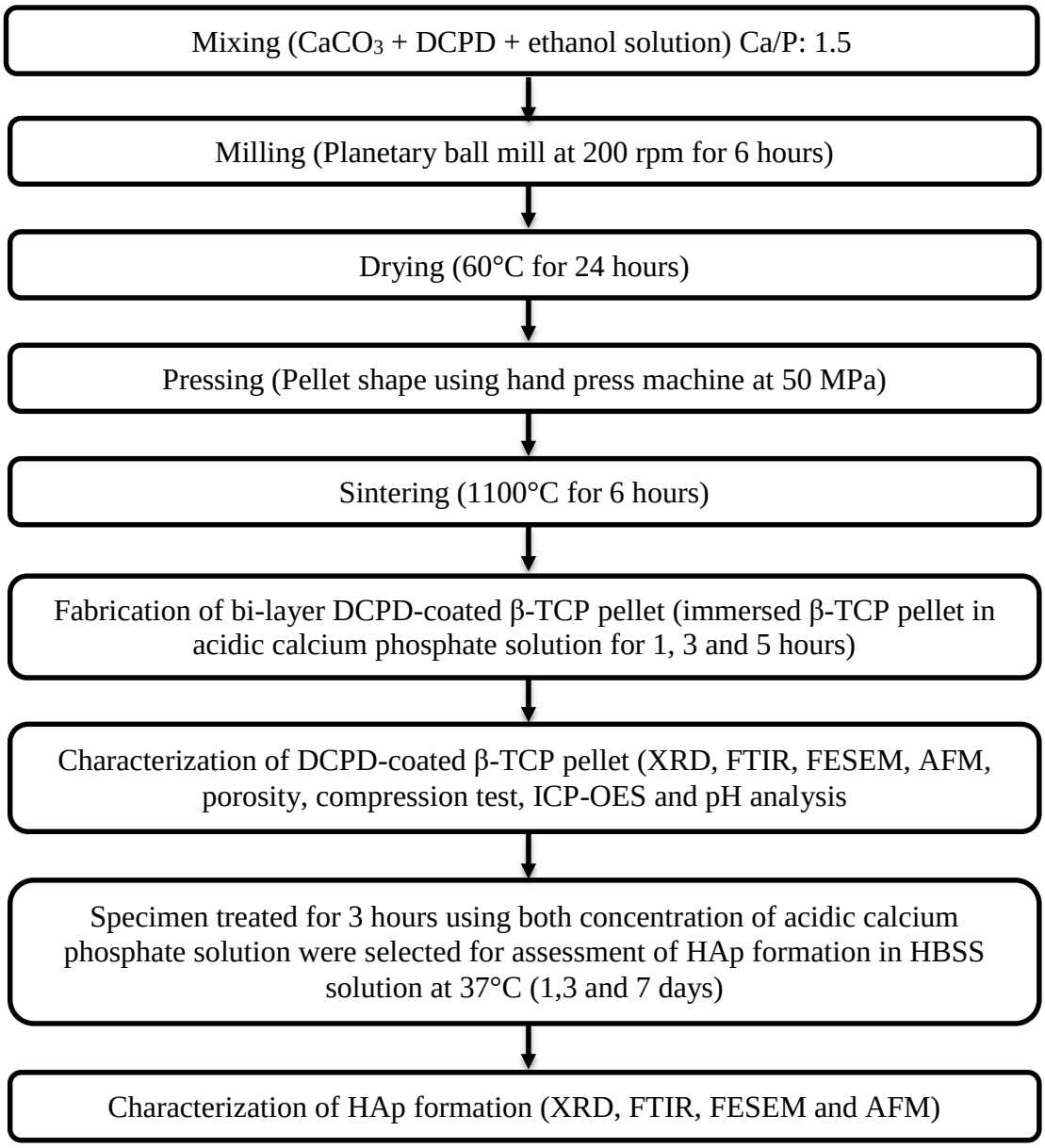

Figure 1.1: The flowchart process of fabricating bi-layer DCPD-coated β-TCP pellet and its characterization.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction of bioceramics as bone substitute materials for clinical application

Bioceramics is a type of ceramics with biological functionality and its classify as the advanced ceramics material, product or component that utilize in wide range of clinical application. Usually, bioceramics is used for repairing and replacing the damaged of bone tissues depending on the application. Since the human and animal bodies consist of bioceramics such as teeth and bone, this bioceramics give an out-standing application to replace various bioceramics parts. Moreover, bioceramics have the properties of biocompatibility and bioactive which helps in the interaction with the surrounding tissues in the body either by supporting tissue growth or by inducing new bone formation. Some of the clinical application of bioceramics that has been widely used are hip prosthesis, cardiac valves and dental implants which due to their great inertness to physiological fluids, having a high compressive strength and give the aesthetic value (Shanmugam & Sahadevan, 2018).

Besides, bioceramics can act as a bioinert ceramics where it remains inactive in mechanical load carrier application (Roy, Bandyopadhyay, & Bose, 2016). In addition, there various types of biological interaction that should be considered between the materials and the body immune system. First, the toxicity of the materials which tissues could die due to the chemical leaching from the ceramics. This reaction usually happens when the tissue are react with implanted material and shows an inflammatory response where it is usually develop around 2 to 7 days after implantation . Second, the biologically inert of the materials which the tissues can

forms a non-adherent fibrous capsule around the implant surface. In other words, introducing a bioinert materials to the body and will not cause any harmful reaction. Third, bioactive materials which the tissue can chemically make a bonding with the surface of implants. The bonding was due to the formation of hydroxyapatite on the materials when immersed in body fluid (Mohamed, El-Aziz, & Breitinger, 2019).

An osteoconductive material is one of the bioactive materials where the material can makes a bond with the hard tissues and stimulate the new bone formation. The bond formed are strong due to the similar apatite layer in host bone (Hench & Jones, 2005). Lastly, the dissolution of implant in which the surface of implant dissolves and allow tissues to fully spread into the space occupied by the implant before. The main bioactivity mechanism of calcium phosphate (CaP) is dissolution and release of ionic products where it is mainly depends on their chemical composition. Studied by Eliaz and Metoki, (2017) mentioned that CaP material such as HAp, TCP and OCP are not easily dissolve in vivo. However, in body fluids, OCP and TCP dissolve much faster than HAp. The statement was proved by Leó and Jansen, (2009) where usually implants made up of HAp are often remains several years in the body and the dissolution are expected to be significant. Table 2.1 has classified various type of bioceramics and its clinical application as a bone substitute.