SYNTHESIS OF CALCIUM PHOSPHATE FROM LOCAL FISH BONE FOR BIOMEDICAL APPLICATION

MAZATUL NADIA BINTI MOHD ZAFRI
B051410284

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Submitted in accordance with requirement of the Universiti Teknikal Malaysia Melaka (UTeM) for the Bachelor Degree of Manufacturing Engineering (with Hons.)

by

MAZATUL NADIA BINTI MOHD ZAFRI

B051410284

951022-11-5356

FACULTY OF MANUFACTURING ENGINEERING

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I hereby, declared this report entitled “Synthesis of Calcium Phosphate from Local Fish Bone for Biomedical Application” is the results of my own research except as cited in reference.

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APPROVAL

This report is submitted to the Faculty of Manufacturing Engineering of Universiti Teknikal Malaysia Melaka as a partial fulfillment of the requirements for the degree of Bachelor of Manufacturing Engineering (Broad-based) (Hons.). The member of the supervisory committee is as follow:

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(Dr. Toibah binti Abdul Rahim)

ABSTRAK

Hydroxyapatite (HA) adalah salah satu daripada bio-bahan yang digunakan secara meluas dalam pelbagai bidang untuk pelbagai aplikasi bioperubatan dan klinikal. Hydroxyapatite kebanyakannya digunakan sebagai alat perubatan untuk penggantian tulang atau penggantian sendi. Pada dasarnya, HA diperolehi melalui proses sintesis yang melibatkan bahan buangan bio yang terdiri daripada beberapa sumber seperti tulang ikan, sisik ikan, dan tulang haiwan. Dalam kajian ini, HA telah disintesis dari tulang ikan yang dikumpulk dari kawasan sekitar. Proses sintesis HA dilakukan melalui rawatan haba serta proses kalsinasi pada suhu yang berbeza dari 600°C hingga 1000°C. HA yang disintesis kemudiannya dibentuk menjadi bentuk padat dengan menggunakan kaedah tekanan dari pelbagai arah dan disinter pada 1250°C. Analisis XRD menunjukkan bahawa fasa utama adalah HA dalam semua serbuk. Selain itu, keamatan serbuk HA yang diperolehi dengan rawatan kalsinasi pada pelbagai suhu meningkat apabila suhu meningkat. Walau bagaimanapun, apabila suhu meningkat lebih daripada 800°C, puncak HA menjadi rendah dan lebih luas kerana HA mungkin berubah menjadi fasa lain; tri kalsium fosfat. SEM menunjukkan bahawa serbuk HA telah dibentuk oleh aglomerasi zarah-zarah kecil. Pensinteran pada 1250°C mengakibatkan penguraian HA kepada TCP. Analisis kekerasan menunjukkan bahawa kekerasan badan padat HA meningkat apabila suhu kalsinasi untuk menghasilkan HA dari tulang ikan meningkat. Kajian ini mendedahkan bahawa sifat serbuk HA awal yang dihasilkan dari tulang ikan yang dikalsinasi pada suhu yang berbeza memainkan peranan penting bagi menetukan sifat atau ciri HA yang padat.

ABSTRACT

Hydroxyapatite (HA) is one of the biomaterials which is widely used in various fields for multiple biomedical and clinical application. Hydroxyapatite is mostly implemented into medical devices as bone graft or joint replacement. Basically, HA is obtained by extraction process which involved the bio waste of few resources such as fish bones, fish scales, and animal bones. In this study the HA was synthesized from the fish bones collected from the local area. The synthesizing process of HA was done via simple heat treatment by calcination at different temperature ranging from 600 °C to 1000 °C. The as-synthesized HA then were used to form dense body using the uniaxial pressing method and sintered at 1250°C. XRD analysis shows that the major phase was HA in all powders. Moreover, the intensity of HA powder obtained by treated by calcination at various temperature was increased as the temperature increased. However, as the temperature was increased more than 800°C, the HA peaks become less intense and broader as HA might transform to other phase; tri-calcium phosphate. SEM reveals that the HA powders were formed by agglomeration of small particles. Sintering at 1250 °C resulted in the decomposition of HA to TCP. The hardness analysis shows that the hardness of dense HA was increased as the calcination temperature to produce HA from fish bone was increased. This study reveal that the property of the initial HA powder which produce from fish bone calcined at different temperature played a significant role for the property of dense HA.

DEDICATION

Dedicated to my beloved mother, father, family

and all my friends.

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HA	-	Hydroxyapatite
TCP	-	Tricalcium Phosphate
β-TCP	-	Beta- Tricalcium Phosphate
CA	-	Calcium
O	-	Oxide
H	-	Hydrogen
P	-	Phosphate
Eq	-	Equation
XRD	-	X-Ray Diffraction
SEM	-	Scanning Electron Microscopy
TGA	-	Thermogravimetric Analysis
DTA	-	Differential Thermal Analysis
FTIR	-	Fourier Transform Electron
HV	-	Vickers Hardness
PSA	-	Particle Size Analyzer
FWHM	-	Full With at Half Maximum
SS	-	Stainless Steel
Co-Cr	-	Cobalt Chromium
Ti	-	Titanium
g	-	gram
cm^3	-	Cubic centimetre
MPa	-	Megapascals (Meganewton per square metre)
h	-	hour
wt%	-	weight percent
kPa	-	Kilopascals (Kilonewton per square metre)
°C	-	Degree Celcius
λ	-	0.154056 nm

θ	-	Effect of calcination temperature on the Vickers Hardness of dense HA
°	-	degree
μm	-	micrometre
mm	-	millimetre
g/ cm^3	-	gram per cubic centimetre
kgf/ mm^2	-	Kilogram-force/square millimeter
nm	-	nanometre

CHAPTER 1

INTRODUCTION

1.1 Background

At present, stainless steel, titanium, and titanium alloys are extensively used as implant materials, which are used in making dental implant, elbow implant, knee implant and shoulder implant for load bearing application (Liu, 2017). However, the increasing use of orthopaedic implants has stimulated interest and concern regarding the chronic, long-term effects of the material used (Sansone, 2013). In spite of the success of the implant surgery, complications such as aseptic loosening of the implants and infection are almost inevitable (Abu-Amer, 2007). The most frequent complication regarding infection is resulting biological response of the body to the material released by the implant (Köse, 2016). Since the bone defect has become a major problem in orthopaedic, the orthopaedic implants will be acknowledge as a great consideration in the biomedical field.

Hydroxyapatite,or (HA, Ca_{10} $(PO_4)_6$ (OH_2) is a synthetic ceramic substance which is composed of calcium and phosphate. Calcium and phosphate is the major mineral portion of normal natural animal bones and teeth. Basically, HA is obtained by extraction process which involved the bio waste of animal bones and teeth; it can be bovine bones, cow teeth, sea shells, cockles, cuttlefish shells, fish scales, snail shells, fish bones, chicken egg shells, corals, and rat bone (Faheem A., Khil, 2009), shells (Sopyan, 2008), corals, etc. The chemical composition of HA is similar to the inorganic components contained in the natural bone matrix and this resemblance has allowed the synthetic HA as the major clinical application as orthopaedics and dental replacement or implant. Recently, multiple of studies and ideas have an ideas have been generated aimed for a better mechanical properties of porous and granules of HA.

Particularly, HA has been used in the biological field, medical practices and clinical application since the year of 1980 primarily due to its excellent biocompatible (Sopyan, 2007). The long term biocompatibility plays the major role for bone substitution in order to ensure no toxic or injuries effect on the biological system in correlation to the existence of new bone substance throughout its lifetime. Moreover, HA is classed as a bioactive materials since it promotes integration with natural living bone; supports migration of connective tissues and ingrowths of bones right up to the implant surface (referred as osseointegration) (Johansson, 2001). Furthermore, various studies explained and potrayed HA as a non- toxic, non-inflammatory, non-immunogenic, very slow biodegrability, as well as good osteoconductive capabilities in order to serve as scaffold on which bone cells can attach and grow.

Basically, all types of bioceramics which have good mechanical properties and suitable for the load bearing application are bioinert. Moreover, HA has a good bioactivity which is able to be implemented in various application in porous, dense and granules form. It can also be used as coating (Sopyan, 2008). According to (Nawawi & Sopyan, 2011). HA has been used in such field as orthopaedics in dense or porous form as the block implants, porous, granules and coating material. Still, the application of HA in multiple field is limited to non-load bearing application due to its fragility and low hardness and flexural strength. Therefore, the development of bioactive dense HA is essential as it plays the major role in the application of load bearing bone implant.

Due to the good biocompatibility and bioactivity properties of HA, it appears to be the most appropriate ceramic material for implantation purposes as it has close similarity with the mineral components and crystal structure to apatite in human skeletal system which is composed of natural bone and teeth and also the ability to bond to the bone (Guo, Li, 2004). The good bone bonding rate and capacity of the HA will be able to help and improve the cementless fixation of orthopaedic prostheses. However, the application or use of HA is receiving such concern due to its lack of strength which is crucial for load bearing as bulk (Nawawi & Sopyan, 2011). HA is brittle and relatively low mechanical strength in contrast with other implant materials such as metals, alloys and high strength ceramics (example: aluminum and zirconium oxides) (Singh, 2001). The best application of HA for the load bearing implant use would be as a coating on another type of material which is genuinely stronger (LeGeros, 1993). Moreover, the HA coatings on metallic implants will eventually combine the mechanical strength of metal with excellent biocompatibility and bioactivity of calcium phosphates.

Dense HA is defined as HA that have porosity less than 5 volume percent (Rui L. Reis, 2003) with the diameter of micropores which is less than 1μm and grain size bigger than 0.2μm. The microporosity of the dense HA is dependent on the sintering period and temperature. Conventionally, the dense HA is obtained by sintering HA powders at a relatively high temperature (M.Piticescu & Zdrentu, 2004). However, nowadays multiples of methods and techniques are being introduced and developed in order to prepare the dense HA. In traditional procedures, HA was prepared by using the uniaxial pressing (L.M.Rodríguez-Lorenzo, 2001) which will compressed the powder into a mould at pressure of 60-800MPa (Umakoshi, 2006) where the HA powder will be mixed with the binder (example : cornstarch) (S.W.K Kweha, 1999), stearic acid in alcohol, polyvinyl alcohol and Duramax. The pressed dense bodies then went through the sintering process normally with temperature ranging from 900ºC to 1300ºC. The temperature higher than this range are not favoured as it may introduced other phases which will cause impurities to the calcium phosphate such as CaO and β-TCP (Nawawi & Sopyan, 2011).

1.2 Problem Statement

Current biomedical devices are mostly produced from the xenogenic, allogenic and autogenic bone. Xenogenic bone is usually from the bovine bone, allogenic bone is from the individual of the same species but with different genetic, autogenic is usually from the same individual body tissue or such material as synthetic biomaterials . Such concern on the transfer of the disease, risks of rejection by the body, lack of immunogenic and osteoinductive by allografts and xenografts bones, as well as confined supply and the vitality of the additional surgery by autografts, the synthetic hydroxyapatite (HA) are to be implemented as advance medical and clinical application and devices. Titanium is one of the mainly used metals for medical application as the load bearing implants. However, in certain period of time, this type of material might reacted with the host tissue as a result of corrosion and wear of. This will cause the toxic effects on the patient. Every type of the bioactive implant should be biocompatible, tougher than the actual living bone, non-toxic and have a modulus comparable to the bone. Based on all the properties listed, Hydroxyapatite is classed as a practical prospect for the bioactive bone implants (Prakasam, 2015). Moreover, the development of HA for biomedical use can be considered as a great option since the cost used is lower, and it is easier to be obtained as it can be extracted from the natural sources such as animal bones which has unlimited supply. The development of HA from the waste material is able to reduce the daily waste which will eventually help to improve the environment.

Recent research development has focused more efforts on the progress of Hydroxyapatite components for the use of high strength bone implants in the form of dense ceramics or as thin films (Prakasam, 2015). There are numerous numbers of applications of dense hydroxyapatite on the transparency aspects and the electrical and mechanical behaviour. Forthcoming applications, the application of the of hydroxyapatite looking as promising based on the bone bonding, advance medical treatment methods and enhancement on the mechanical strength of the artificial bone grafts. In order to optimize the strength, the powdered HA need to be densified quickly in order to ensure the minimal grain growth (Prakasam, 2015).

1.3 Objectives

This study embarks on the following objectives:

i. To prepare HA powder from fishbone waste by using heat treatment technique.
ii. To characterize the properties of the as-prepared HA powder from the waste fishbone by heat treatment at different temperatures.
iii. To develop HA dense bodies via uniaxial pressing using HA synthesize from fish bones.
iv. To characterize physical and mechanical properties of the prepared dense HA.

1.4 Scope of project

Substantively, the project activities, progress and outcomes will be well described in further detail in proceeding chapters. It is to theoretically and practically study on the preparation of porous HA sample from bovine bone with excellence biomedical properties:

i. Process - Heat treatment method (Easy, less preparation needed, less complex)
ii. Fish - Freshwater fish (Pangasianodon hypophthalmus)
iii. The study will emphasized on the attempts to obtain characteristics, physical and mechanical properties of the HA samples.
iv. The characterization of HA powder and dense body will be conducted via Scanning Electron Microscope (SEM), X-ray Diffraction (XRD), and Particle Size Analyzer (PSA).
v. The Vickers Hardness test will be carried out in order to study the hardness of dense HA body with different calcination temperature.
vi. At the end of the experiment, the data on characterization, physical and mechanical properties of HA powder (Calcined at different temperature; 600°C - 1000°C) and dense HA body (Calcined at different temperature; 600°C - 1000°C and sintered at 1250°C) will be compared.

CHAPTER 2

LITERATURE REVIEW

2.1 Biomaterial

Biomaterials field has become an important area as these materials are able to enhance the quality and longevity of the human life while obtaining multi-million dollar profit from the technology and science related to this field. (Ti based biomaterials, the ultimate choice for orthopaedic implants – A review). Biomaterials can be classified as any substances or the combination of the substances, excluding drug which have been engineered naturally or synthetically in order to be used to enhance human health (O. Hollinger, 2006) in clinical field by acting as medical devices which interacts with the biological systems such as artificial bone, organs, tissue implants as well as prostheses. Biomaterials can be either natural or synthetic (Bose**,** 2017**)**.

The growth of multiple classes of biomaterials such as titanium, titanium alloys, stainless steel, and cobalt–chromium alloys (Gotoh, 2004), ceramics which can be aluminum oxide, carbon, calcium orthophosphates and glass–ceramics (Kokubo, 2008), polymers which can be silicon, polymethyl methacrylate, polylactide, polyurethane, and ultra-high molecular weight polyethylene and also composites which can be ceramic coating on metal implants, or ceramic-reinforced polymers (Barrère, 2008) for clinical practice have cause a sudden increase in tissue engineering.

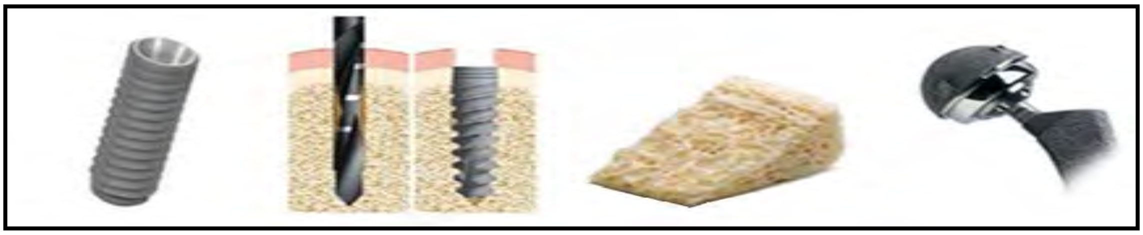

Figure 2.1: Various types of biomedical devices made from various types of biomaterials; Orthopedic implants (a) Spinal implants (b) Orthobiologics (c) Reconstructive joint replacement for knees and hips (Sopyan, 2009).

Generally, biomaterials can be classified into three types which are naturally derived materials (example collagen and alginate), acellular tissue matrices (example bladder submucosa and small-intestinal submucosa), and synthetic polymers [example polyglycolic acid, polylactic acid, and poly(lactic-co-glycolic acid)] which have proved to be beneficial in the reforming of a number of genitourinary tissues in animal models (Lee, 2014). Currently approved and commonly used metallic biomaterials include stainless steels, titanium and cobalt–chromium-based alloys. As stated in (Barrère, 2008), biomaterials are all such special materials which are applied in medical implants and defined as materials which are intended to interface with biological systems to treat or replace any tissue, organ, or function of the body. New materials to repair or replace human skeletal joints (e.g. hip, knee, shoulder, ankle, fingers) are being introduced as materials scientists and engineers develop better understanding of the limitations of current joint replacement technologies (Kunčická, 2017).

2.1.1 Classification of Biomaterial

In reality, none of the biomaterials have completely fulfilled all the requirements for the best implant devices; each material has come up with its own strength and limitations. Thus, a proper selection of material within all those types of biomaterials must be conducted and in order to improve the development of future application and function of