SYNTHESIS AND CHARACTERIZATION OF HYDROXYAPATITE DERIVED FROM FISH BONE BY HEAT TREATMENT

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This report is submitted in accordance with requirement of the University Teknikal Malaysia Melaka (UTeM) for Bachelor Degree of Manufacturing Engineering (Hons.)

by

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Date : 30 June 2022

APPROVAL

This report is submitted to the Faculty of Manufacturing Engineering of Universiti Teknikal Malaysia Melaka as a partial fulfilment of the requirement for Degree of Manufacturing Engineering (Hons). The member of the supervisory committee is as follow:

………………………………………………

(DR. TOIBAH BT ABD RAHIM)

ABSTRAK

Matlamat kajian ini adalah untuk mendapatkan biomaterial daripada sisa tulang ikan menggunakan proses pengkalsinan. Sejak beberapa dekad, biomaterial hidroksiapatit (HA) telah diterokai untuk aplikasi perubatan. Tulang ikan merupakan salah satu bahan yang boleh digunakan untuk menghasilkan biomaterial HA. Tambahan pula, memandangkan sisa tulang ikan banyak terdapat dalam populasi, ia merupakan sumber bahan mentah kos rendah dan kaedah terbaik untuk mengurangkan pengeluaran sisa daripada industri perikanan. Dalam kajian ini, serbuk HA adalah sintesis daripada tulang ikan Euthynnus Affinis yang dikumpul sendiri daripada sisa. Tulang ikan akan dikalsinkan pada suhu berbeza antara 600ºC hingga 1200ºC untuk menghasilkan serbuk HA. Kesan suhu pengkalsinan dan masa rendaman ke atas HA terhasil yang disediakan daripada sisa tulang ikan akan dikaji menggunakan XRD, FTIR, PSA, dan SEM. Dijangkakan bahawa apabila suhu pengkalsinan dan masa rendaman meningkat, kehabluran dan saiz zarah serbuk HA meningkat dengan penurunan keliangan akibat pembentukan struktur mikro yang lebih tumpat. Jalur sempit OH^- dan PO_4^{3-} juga diperhatikan oleh FT-IR apabila suhu pengkalsinan meningkat, yang mengesahkan pembentukan fasa HA. Penemuan daripada PSA telah mendedahkan bahawa stuktur HA bergumpal pada skala bersaiz mikron, manakala SEM telah membuktikan zarah adalah dalam keadaan bergumpal. Tambahan pula, hasil kajian menggunakan PSA menunjukkan bahawa semakin tinggi suhu pengkalsinan pada masa perapan selama 1 sehingga 3 jam telah meningkatkan taburan saiz zarah. Kesimpulannya, penemuan in adalah menjanjikan penggunaan aplikasi dalam perubatan pada masa hadapan yang melibatkan pemulihan tulang.

ABSTRACT

The aim of this study is to obtain biomaterial from fish bone waste using calcination process. Since decades, hydroxyapatite (HA) biomaterials have been explored for medical applications. Fish bones are one of the materials that may be utilized to produce HA biomaterial. Furthermore, as fish bones waste is abundant in the population, it is a low-cost source of raw materials and an excellent method to reduce waste production from fisheries industries. In this study, HA powder was synthesised from Euthynnus Affinis fish bone which was self-collected from kitchen waste. The fish bones were calcined at different temperatures between 600ºC to 1200ºC to produce HA powder. The effect of calcination temperatures and soaking times (1 & 3 hours) on the as-produced HA which prepared from the fish bone waste were studied using XRD, FTIR, PSA, and SEM. The XRD analysis confirmed that when the calcination temperature and soaking time was increased, the crystallinity and particle size of the HA powders was increased with decreased of porosity due to formation of denser microstructure. Narrow band of OH^- and PO_4^{3-} are also observed by FT-IR when the calcination temperature is increased, which confirmed the formation of HA phase. PSA reveals that the HA structure was agglomerated in micron-sized regions, while SEM confirms that the agglomerated particles were composed of nanoparticles. Furthermore, PSA results show that increasing the calcination process soaking time from I to 3 hours resulted in a significant increase in particle size distribution. In conclusion, these findings are promising for future medical applications involving bone restoration.

DEDICATION

My beloved parent,

Muhammad Zainudin bin Mohamed Kamal

Kartika binti Abd Kadir

My respected supervisor, Dr. Toibah bt Abd Rahim

My adored siblings,

Farah Aqila binti Mohamed Zainudin

Farah Adiba binti Mohamed Zainudin

Muhammad Zubair bin Mohamed Zainudin

Farah Arisya binti Mohamed Zainudin

For giving me guidance, courage, support, understanding, cooperation, and kindness

Thank You Very Much

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α-TCP	-	Alpha Tricalcium Phosphate
β-TCP	-	Betta Tricalcium Phosphate
BCP	-	Biphasic Calcium Phosphate
FA	-	Fluorapatite
FTIR	-	Fourier Transform Infrared Spectroscopy
HA	-	Hydroxyapatite
PSA	-	Particle Size Analyser
SEM	-	Scanning Electron Microscopy
TCP	-	Tricalcium Phosphate
TTCP	-	Tetra Calcium Phosphate
TG/DTA	-	Thermogravimetry and Differential Thermal Analysis
XRD	-	X-Ray Diffraction

LIST OF SYMBOLS

α	-	Alpha
β	-	Betta
ºC	-	Degree Celsius
θ	-	Theta
λ	-	Lambda

CHAPTER 1

INTRODUCTION

This chapter provides a detailed description of the research project. This chapter includes the research background, problem statement, objectives, research scopes, research rationale, research technique, and project report organization.

1.1 Background

Every year, millions of cases of bone graft and bone implantation surgery were performed by orthopaedic surgeon in medical. According to Zhu et al. (2017), approximately 2.2 million cases of bone implantation surgery are conducted worldwide per year which caused by severe trauma, an infection, tumour removal, and other congenital illnesses. Although bone has its own potential for self-healing/regeneration and can cure any tiny bone defect, bigger bone defects are unlikely to be healed by the bone itself. Therefore, bone implantation surgery is required to help larger bone defects by making a scaffold for the bone to heal and grow. Despite significant progress in bone tissue transplant research, it remains difficult to achieve success in medical applications due to a variety of factors. The main issues are the bioactivity of the replacement materials used due to rejections in the human system that can cause harm (Shi et al., 2018). Above, the measures of restoration of bone defects usually involve the use of a synthetic biomaterials.

Human bones are basically composed of 20% of collagen fibrils, 69% of nano-sized crystalline inorganic phases, which resemble of hydroxyapatite (HA), and 9% of water

(Anitha & Pandya, 2014). Therefore, HA is a critical calcium phosphate-based substance that closely replicates the mineral component of real bones. HA has been used in medical field over 50 years in bone implantation material (Akram et al., 2014). HA is made up with β-tricalcium phosphate (β-TCP) also called biphasic calcium phosphate (BCP), was performed better in osteoconductivity that may stimulate fast bone regeneration and reconstruct the hard tissue. Besides, the molar ratio of HA itself, which is 1.67, makes it biocompatible and bioactive to human bone (Boutinguiza et al., 2012; Akram et al., 2014). HA has been the most essential component to human bone (Piccirillo et al., 2013).

Hydroxyapatite can be obtained by various process or method such as hydrothermal method, solvothermal method, and homogenous precipitation method. In general, HA can either be obtained from organic or inorganic (Zhu et al., 2017) that produced from natural by-product or waste materials, or through chemical synthesis (Piccirillo et al., 2013). As known, main component of natural bone (HA) can be formed organically from natural or biological resources such fish bone, eggshells, bovine bone, and others animal bone (Akram et al., 2014). In this study, simple heat treatment is used to produce HA from fish bone waste.

1.2 Problem Statement

Many efforts have been made in recent decades to find alternative natural sources for biomaterials. The potential of HA extracted from animal skeletons, such as bovine, chicken, and fish bone. Every year, almost 91 million tonnes of fish and shellfish are caught throughout the world (Boutinguiza et al., 2012). Meanwhile, Malaysians consume one of the greatest amounts of fish per capita in the world, at 56.8 capita per year. Furthermore, (Piccirillo et al., 2013) also mentioned that only half of the catch is usable for human consumption, with the remaining being wastes or by-products. As known, excessive wasted raw resources have an undesirable influence on the environment. The solid waste that collected from the fisheries industries are usually dumped in the landfills which can causing contamination to the land and water resources as the wastes are high in toxicity. Besides, the recycling process of waste in order to get valuable material resources is gaining attention across the world because of the increasing amounts of waste products that needs to be disposed (Piccirillo et al., 2013).

In order to reduce the excessive waste products that produced from fisheries industries or food industries, fish bones that would otherwise be wasted might be used to provide a low-cost source of calcium phosphate that can be used in medical fields. Calcium phosphates are recognised for their usage as biomaterials in several field. The calcium phosphate that focuses on this study is hydroxyapatite (HA). Since the molar ratio is same between calcium phosphates and hydroxyapatite, which is 1.67 (Boutinguiza et al., 2012), these biomaterials are majorly used in medical field especially in orthopaedics and orthodontics due to the substance is the most similar to the inorganic component of bones and teeth. Moreover, biphasic calcium phosphate (BCP), which is made up of Hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP), was expected to perform better osteoconduction and osteoinconduction in bone grafting (Zhu et al., 2017). The issue is that producing synthetic calcium phosphate is expensive and difficult to generate. Hence, using waste fish bones material will critically reduce the cost of getting raw materials and much safer because of using an organic waste material.

1.3 Objectives

The objective of the research are as follows:

i. To obtain hydroxyapatite powder from fish bone waste by heat treatment process at different calcination temperatures and different soaking time.
ii. To investigate the effect of different temperatures and soaking time on the properties of as-produced hydroxyapatite derived from fish bones waste using XRD, FTIR, PSA, and SEM.

1.4 Scope

Fish bones contains numerous valuable organic and inorganic component. Mainly, in this report is to derived hydroxyapatite (HA) powder from an *Euthynnus Affinis* fish bone as the raw material. The methods for extracting HA from fish bones have been thought up, using simple heat treatment at the temperature of 600ºC, 800ºC, 1000ºC, and 1200ºC, with different soaking time for the fish bones for 1 and 3 hours. The as-prepared HA are characterized based on the microstructure, crystallinity, phase transformation, and particle size, using XRD, PSA, SEM, and FTIR.

1.5 Rationale of Research

The rationale of this research is to encourage the synthesis of hydroxyapatite (HA) from waste product fish bones due to its massive waste production in landfills that can causes threats to environment. This approach not only reduces waste generation in the environment, but it also benefits mankind because the synthesis process of HA may very well be employed in medical fields to cure people. For the cases of injury that required bone replacement is where HA play its role because one of its advantages is that it may be utilised as a coating material to initiate the interaction between human bones and implant material because the substance is the most comparable to the inorganic component of bones and teeth. Furthermore, the process of synthesis HA is through heat treatment process that will be defined the results based on different heating temperature. Hence, the goals for this research will be used as future references tool for studies on the production of HA from discarded fish bones or organic wastes.

1.6 Project Report Arrangement

The flow organization of this report such; Chapter 1 will include the research background, problem statement, objectives, research scope, research rationale, research technique, and project report organization. Chapter 2 broad a topic about biomaterials and the classification of biomaterials which is including metallic, polymer, and ceramic. The purpose and applications of biomaterials and bio ceramic as well as the response of these materials to body system. Further, the processes of obtaining biomaterials from natural resources such fish bone, fish scales, shellfish, and other animal bone. Chapter 3 covers the method of preparing, analysing and synthesis of biomaterial such flow chart, material, sample preparation, characterization method, and analysis.

CHAPTER 2

LITERATURE REVIEW

This chapter will broad a topic about biomaterials and the classification of biomaterials which is including metallic, polymer, and ceramic. The purpose and applications of biomaterials and bio ceramic as well as the response of these materials to body system. Further, the processes of obtaining biomaterials from natural resources such fish bone, fish scales, shellfish, and other animal bone.

2.1 Introduction of Biomaterials

There has been significant progress in the development of biomaterials in previous years, including numerous ceramic materials for bone repair and reconstruction. The key drivers in this trend appeared to be improvements in life expectancy as well as societal commitments to offer a higher quality of life (Dorozhkin, 2009). The word “biomaterials” has been given a variety of meanings. Biomaterials, often known as biomedical materials, are synthetic or natural materials that are intended to replace components of a living system or to function in close proximity to living tissues. According to Dorozhkin (2009), biomaterial is a substance that has been created to adopt a shape that, alone or as part of a complex system, that utilised to guide the course of any therapeutic or diagnostic operation in human or veterinary medicine by control of interactions with components of biological systems.

The strength of biomaterials is to sustain in a biological environment without causing damage to the environment or being damaged in the process distinguishes them from other types of materials. Therefore, biomaterials are exclusively connected with the health-care industry and must interact with tissues or tissue components. The goal is to ensure proper biological interaction between the implanted biomaterial and the host's live tissues (Dorozhkin, 2009). In bone structure normally consist of calcium-based biomaterials. The primary technical rationale for the preference for calcium-based substances is that calcium phosphates, of which hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$, HA] (Brostow et al., 1999). Hydroxyapatite can be made from synthetic or organic materials. Despite the fact that both forms of biomaterials are bioactive and biocompatible in nature and also well suited for in-vitro applications, the primary disadvantage of biomaterials produced from inorganic calcium (Ca) and phosphate-based sources is the high expense of the synthetic process (Akram et al., 2014). Furthermore, HA generated from natural organic sources is non-stoichiometric, which might be owing to trace amounts of ions present in natural organic sources.

In addition, this report is focusing on extracting biomaterials which is hydroxyapatite from fish bone waste. HA is the most important component of human bone, and it is commonly used in the medical field due to its high biocompatibility that helps bone growth. It is a well-known bio ceramic material that is widely used in various medical applications as a promising material for healing damaged bones due to its biocompatibility and bioactivity, as well as its inorganic composition similar to that of natural bone (Shi et al., 2018). In clinical or medical, larger bone defects can be healed by using bone scaffold as shown in Figure 2.1. Moreover, the characteristic of biomaterials of HA can be improve by combining HA with calcium-based materials, also known as biphasic calcium phosphate (BCP). HA is frequently can be found with β-tricalcium phosphate (β-TCP). Despite the latter has lower bioactivity but it enhanced the resorption capacity. Besides, the concentration of HA can change components in human bones, as well as their biocompatibility and implantability (Piccirillo et al., 2013).

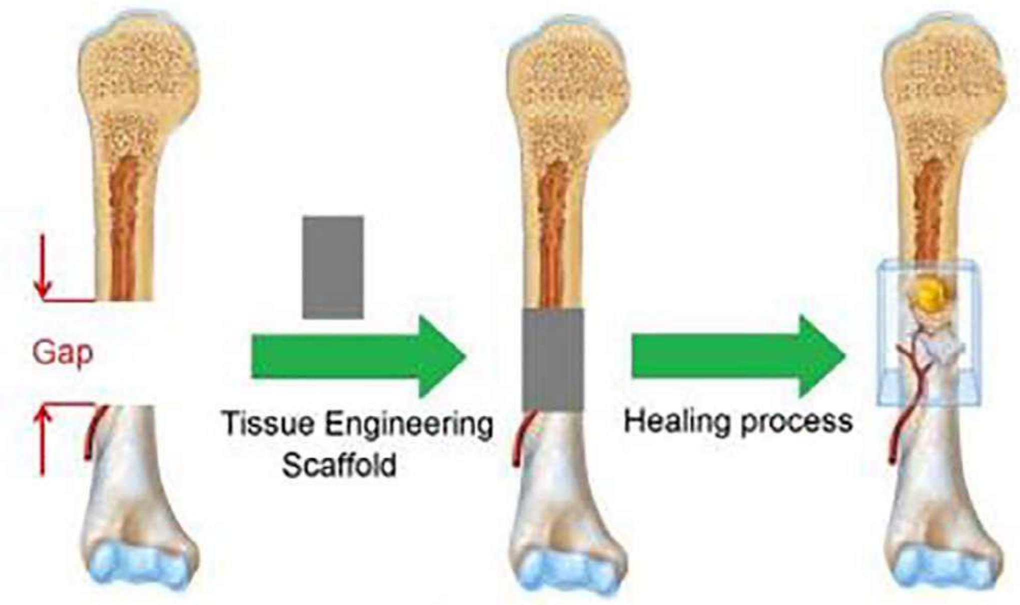

Figure 2.1: Bone scaffold mechanism (Kalsi et al., 2021)

2.1.1 Classification of Biomaterials

Biomaterials are materials that are employed in biological environments and must remain in such environments (Gautam et al., 2021). There are various biomaterials with varied compositions to construct scaffolds that imitate the extracellular matrix (ECM) to support the generating new tissue bone (Kalsi et al., 2021). Basically, biomaterials are categorized as metallics, polymers, and ceramics (Gautam et al., 2021). Brostow et al. (1999) mentioned that biomaterials with a diverse set of properties are required for surgical applications. In these sections, the various biomaterials will be discussed generally.