UNIVERSITI TEKNIKAL MALAYSIA MELAKA

DEVELOPMENT OF HYDROXYAPATITE/CHITOSAN COMPOSITE POWDER VIA MECHANICAL MILLING METHOD

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

by

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APPROVAL

This report is submitted to the Faculty of Manufacturing Engineering of UTeM as a partial fulfillment of the requirements for the degree of Bachelor of Manufacturing Engineering (Engineering Materials) (Hons.). The member of the supervisory committee is as follow:

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ABSTRAK

Hydroxyapatite (HA) adalah kalsium fosfat yang berasaskan seramik yang telah digunakan sejak 20 tahun yang dulu didalam bidang perubatan dan pergigian. Komposis kimia yang terdapat dalam HA adalah sama dengan tisu tulang bukan organik serta sifat-sifat mekanikal adalah sama dengan tulang mineral. Namun, penggunaan HA hanya dapat menyokong beban yang ringan kerana ia rapuh. Maka adalah penting untuk meningkatkan sifat mekanikal HA tanpa menjejaskan sifat biologikal, keserasian bio dan ostreokonduktif yang dimilikinya. Untuk tujuan tersebut, adalah penting untuk menggabungkan HA dan bahan biopolymer maka ia boleh digunakan sebagai penggantian untuk tulang. Diantara kebanyakan bahan polimer, Chitosan (CS) mempunyai ciri yang menarik seperti tidak toksid, boleh terbiodegrasikan dan fleksibel. Dalam kajian ini, campuran HA/CS akan dihasilkan melalui kaedah pengisaran mekanikal untuk menghasilkan komposit yang seragam. Kepekatan bahan pengisi CS yang dimasukkan adalah sebanyak 5, 10 dan 20 wt.%. Kesan kepekatan CS dalam HA akan dikaji menggunakan XRD untuk analisis fasa, dan sifat mekanikal seperti kekerasan, ketumpatan dan kekuatan mampatan diuji melalui ujian kekerasan Vickers, ujian ketumpatan dan ujian kebolehmampatan. Pada analisis XRD, fasa HA dan CS masih wujud setelah melalui proses persinteran dan untuk sifat mekanikal kekerasan dan ketumpatan HA- 20 wt.% CS telah menunjukkan keputusan yang optimum. Walaubagaimana pun, untuk analisi kekuatan mampatan, HA-5 wt.% telah merekodkan keputusan yang terbaik berbanding komposisi komposit yang lain.

ABSTRACT

Hydroxyapatite (HA) is a calcium phosphate based ceramic that has been used for over 20 years in medicine and dentistry. The chemical composition of HA materials is same like bone tissue's inorganic constituent and its mechanical properties are similar to mineral bone. Still, the use of HA made prosthesis supports only lighter loads as it is fragile. So it is important to enhance the mechanical properties of HA without affecting its biological, biocompatibility and ostreoconductive nature. For this purpose it is important to entrap the HA with biopolymeric material so that it can be used as a superior replacement for bone and also can be used as bone filler material. Among organic polymers, Chitosan (CS) has attractive features as bone substitute such as low toxicity, biodegradability and high flexibility. In this study, a mixture powder of HA/CS will be conducted using mechanical milling process to produce homogenize HA/CS composite powder. The concentration of CS filler added to the HA powder are 5, 10 and 20 wt.%. The effect of CS concentration in HA were investigated using XRD for phase analysis and the mechanical properties such as hardness, density and compressive strength were evaluated by Vickers microhardness, density and compression testing. In XRD analysis, HA and CS phase is still exist after the sintering process and for mechanical testing the optimum microhardness and density were represented by HA-20 wt.% CS. However, under compressive strength HA-5 wt.% CS recorded the best result compared to others composite composition.

DEDICATION

Dedication to my beloved parents, family,
and all supporters.

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First of all, in a humble way I wish to give all the Praise to Allah, the Almighty God for with His mercy has given me the strength and time to complete this report.

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LIST OF ABBREVIATIONS

ASTM	-	American Standard Testing Material
BCC	-	Body Center Cubic
CS	-	Chitosan
DD	-	Degree of Deacetylation
DNA		Deoxyribonucleic acid
HA	-	Hydroxyapatite
HCP	-	Hexagonal Closed Packed
Hv	-	Hardness Vickers
PHB		Polyhydroxybutyrate
UK	-	United Kingdom
XRD	-	X-Ray Diffraction
MPa	-	Megapascal
µm	-	Micronmeter
gm	-	Gram
nm	-	Nanometer
N	-	Newton
°C	-	Degree celcius
HV	-	Hardness Vickers
Wt. %	-	Weight percentage
kgf	-	Kilogramforce
g/cm^3	-	Gram per cube centimeter
N/mm^2	-	Newton per square milimeter

CHAPTER 1

INTRODUCTION

1.1 Background

There is a necessity for replacing bone substance which has been lost due to traumatic or nontraumatic events (Suchanek *et al.,* 1998). The implanted biomaterial must have certain desired properties in order to achieve a satisfactory result and to have an appropriate host response at the hard tissue implantation site. The microstructural and mechanical properties of the bone must be thoroughly understood for the successful preparation of candidate hard tissue implant biomaterials in order to mimic the natural bone structure.

A biomaterial is any substance or combination of substances, which can be used for any period of time, as a whole or as a part of a system for use in the human body to measure, restore, and improve physiologic function, and enhance survival and quality of life. Typically, inorganic (metals, ceramics, and composites) and polymeric (synthetic and natural) materials have been used for such items as artificial heart-valves, (polymeric or carbon-based), synthetic blood-vessels, artificial hips (metallic or ceramic), medical adhesives, sutures, dental composites, and polymers for controlled slow drug delivery. Polymers have very low mechanical strength compared to bone, metals have superior mechanical properties but they are very corrosive, and despite their other desired properties such as wear resistance, biocompatibility and hardness, ceramics are brittle and have low fracture toughness.

Biomaterials must be compatible with body in order to exhibit their function properly. The use of incompatible materials as medical implants in the body may induce unfavourable immune reactions, undesirable interactions with blood and other body fluids as well as damaging the genetic material at the chromosomal and DNA levels.

Bone consists of 69 wt. % calcium phosphate (mainly hydroxyapatite (HA)), 21% collagen, 9%water and 1% other constituents. It has a composite nature which is composed of mainly ceramic and polymeric components with a complex hierarchical microstructure difficult to imitate which gives most of the superior mechanical properties to bone. Extensive research has been conducted on bone substitute composite materials composed of mainly hydroxyapatite and a polymer (chitosan).

Hydroxyapatite ($Ca_{10}(PO_{4})_{6}(OH)_{2}$) has excellent bioactivity, biocompatibility, non-toxicity and osteoconductivity properties but also has low toughness. Chitosan (CS), deacetylated form of chitin, is a natural polymer found in vast amounts in crustaceans. It is biocompatible and bioresorbable/biodegradable. It is non-toxic and easily soluble in dilute weak organic acids. The recent research on chitosan/hydroxyapatite composites which are partially biodegradable indicate that this behaviour of the composite may even be an advantage. New bone may intergrowth around the hydroxyapatite particles when the polymer matrix is resorbed. Extensive research on relatively new chitosan/hydroxyapatite composites has been conducted recently (Zhao *et al.,* 2007).

In this research, HA/CS composite powder with varian composition are produced by mechanical milling. XRD, compression, densitometer and hardness test machine will be used to analyze and characterize the composite powders.

1.2 Problem Statement

Metallic implants are widely used in many treatments and are fairly successful. However, they do not provide the optimum therapy due to their short comings such as stress shielding during post-healing, chronic inflammation caused by corrosion, and fatigue and loosening of the implant. As a result, a second surgery is often required to remove the metallic implant after healing, and it increases the risk of the operation and the expense to the patient. Degradable polymeric implants eliminate the need for a second operation and can prevent some of the problems associated with stress shielding during post-healing, and can also be used simultaneously to deliver therapeutic drugs to treat infections or growth factors to accelerate new bone growth. Chitosan (CS), was suggested as an alternative polymer for use in orthopedic applications to provide temporary mechanical support the regeneration of bone cell ingrowth due to its good biocompatible (Vande *et al.,* 2002), non-toxic, biodegradable, and inherent wound healing characteristics (Lee *et al.,* 2002). According to Sabokbar *et al.,* (2001) HA was used in various biomedical fields such as dental material, bone substitute and hard tissue paste. HA can accelerate the formation of bone-like apatite on the surface of implant. CS can be utilized in combination with other bioactive inorganic ceramics, especially HA to further enhance tissue regenerative efficacy and osteoconductivity. Incorporation of HA with CS, the mineral component of bone, could improve the bioactivity and the bone bonding ability of the HA/CS composites (Wang *et al.,* 2002). The aim of this work was to prepare homogenous HA/CS composite via mechanical milling method, in which the matrix HA is milled with the additional various of CS content in order to identify the effect of CS on mechanical properties of produced composite. It can be expected that by increasing chitosan, the aggregation of particles enhance and improve the compatibility among CS filler and HA matrix.

1.3 Objective of Research

The main objectives of this research are:

1. To prepare composite biomaterials by using chitosan and hydroxyapatite powder through mechanical milling.
2. To characterize and evaluate the physical and mechanical properties of the produced composite powder.

1.4 Scope of Research

In this study, HA (Merck, US) and CS (Sigma Aldrich, UK) powder were used in preparation of HA/CS composite. This research is focused on the effect of enhancement various component of chitosan on HA/CS composite. There are 3 samples of HA/CS were produced and the concentration of CS powder added to the HA powder are 5, 10 and 20 wt. %. HA and chitosan powder will be blended using ball milled machine in order to produce homogenous HA/CS composites powder. The HA powder is mechanically mixed with the chitosan powders for 5 hours in a planetary mill using Al_2O_3 balls and a jar, resulting in the preparation of the homogenous HA/CS powder mixture. Thereafter, the mixture of powder were sieved using sieve shaker 40 μm to obtain smaller HA/CS particles size. In fabricating process, for powder compaction, die pressing method is used with pressures of 3.5 tons to form HA/CS pellet with the diameter size of 13 mm and thickness is 6 mm. The density of the HA/CS pellet produced from die pressing is measured before undergo sintering process. Then samples were sintered in electrical furnace. The samples were sintered at 1000°C heating temperature with heating rates of $5°\mathrm{Cmin}^{-1}$ and soaking time 60 minutes.

After the sintering process, all HA/CS sample will be analyzed. X-Ray Diffractometer (XRD) also will be used in order to identify the phase composition of fabricated HA/CS composite. Furthermore, for mechanical properties, hardness, density and compression test will be conducted by using microhardness Vickers, densitometer and universal testing machine which are available in FKP lab.

CHAPTER 2

LITERATURE REVIEW

This chapter discussed on structure composite material. The contents are focusing on introduction, structure, application and advantage from the study.

2.1 Introduction of Hydroxyapatite

According to Soley (2012), Hydroxyapatite is a mineral. It is a naturally occurring form of calcium apatite with the formula $Ca_5 (PO_4)_3(OH)$, but is usually written $Ca_{10} (PO_4)_6(OH)_2$ to denote that the crystal unit cell comprises two entities. Hydroxyapatite is the hydroxyl endmember of the complex apatite group. The OH- ion can be replaced by fluoride, chloride or carbonate. It crystallizes in the hexagonal crystal system. It has a specific gravity of 3.08 and is 5 on the Mohs hardness scale. Pure hydroxylapatite powder is white. Naturally occurring apatites can however also have brown, yellow or green colorations, comparable to the discolorations of dental fluorosis.

Seventy percent of bone is made up of the inorganic mineral hydroxylapatite. Carbonated-calcium deficient hydroxylapatite is the main mineral of which dental enamel and dentin are comprised. Hydroxyapatite crystals are also found in the small calcifications (within the pineal gland and other structures) known as corpora arenacea or 'brain sand'.

Hydroxyapatite is the major constituent of the inorganic component of bone. Although similar to natural bone in many respects, synthetic HA vary from the natural bone in that they tend to contain larger and more uniform crystals with a more homogenous composition than those found in natural bone. Due to this more crystalline and organized structure and since hydroxyapatite is resorbed by foreign body giant cells, which stop ingesting once 2 to 10μm of hydroxyapatite has been consumed, synthetic HA tend to resorbd extremely slowly in the body, often taking many decades to resorb fully (Kurtz *et al.,* 2006).

Hydroxyapatite can be found in teeth and bones within the human body. Thus, it is commonly used as a filler to replace amputated bone or as a coating to promote bone ingrowth into prosthetic implants. Although many other phases exist with similar or even identical chemical makeup, the body responds much differently to them. Coral skeletons can be transformed into hydroxyapatite by high temperatures; their porous structure allows relatively rapid ingrowth at the expense of initial mechanical strength. The high temperature also burns away any organic molecules such as proteins, preventing graft-versus-host disease (GVHD) and rejection.

2.1.1 Hydroxyapatite Properties and Applications

There is a wide variation of mechanical properties of synthetic calcium phosphate as given in Table 2.1. This variation of properties is the result of the variation in the structure of polycrystalline calcium phosphates, in turn the result of differences in manufacturing processes. Depending on the final firing conditions, the calcium phosphate can be calcium hydroxyapatite or β-whitlockite. In many instances, however both types of structure exist in the same final product.

Polycrystalline hydroxyapatite has a high elastic modulus (40-117GPa). Hard tissues such as bone, dentin, and dental enamel are natural composites that contain hydroxyapatite as well as protein, other organic materials, and water. Enamel is the stiffest hard tissue with an elastic modulus of 74 GPa and it contains the most mineral. Dentin (E=21GPa) and compact bone (E=12-18GPa) contain comparatively less mineral. The poisson's ratio for the mineral or synthetic hydroxyapatite is about 0.27, which is close to that of bone (≈0.3) (*Park et al.,* 1992)

Hydroxyapatite (HA) is a bioactive ceramic material with high bioaffinity, biocompatibility and osseoconductivity which is the main constituent of bones and teeth. Natural HA has the advantage that it inherits some properties of the raw material such as composition and structure. Properties of HA have found useful application in low-load bearing porous implants and coatings of metallic implants. Bioactivity and acting as a template for forming and growing of the surrounding bone tissues make HA an excellent choice for coating of the metallic implants.

After implantation of prostheses, a close surface contact between the metallic prosthesis and the surrounding bone tissue is needed for subsequent bone ingrowths. Coating of the metallic implants with bioactive HA leads to a rapid bonding between hydroxyapatite and surrounding bone tissue. Application of HA as a coating of the metallic implants combines the strength and toughness of the substrate with bioactive characteristic of HA which can induce the surrounding bone tissue ingrowths and future formation of chemical bonding. Further, the presence of HA coating can improve corrosion resistance of the coated implant in human body which can reduce the metallic ion release and also promotes fixation via chemical bonding (Nayar *et al.,* 2006).

Table 2.1 Physical Properties of Calcium Phosphate

Properties	Value
Elastic modulus (Gpa)	40 - 117
Compressive strength	294
Bending Strength (Mpa)	147
Hardness (Vickers, Gpa)	3.43
Poisson’s ratio	0.27
Density (theoretical, g/cm^3)	3.16

2.1.2 Crystal Structure of Hydroxyapatite

Calcium phosphate can be crystallize into salt, hydroxyapatite and β-whitelockite depending on the Ca/p ratio, presence of water, impurities and temperature. In a wet environment and at lower temperature, ($< 900^{\circ}C$) it is more likely that the hydroxyl apatite will form, whereas in a dry atmosphere and at higher temperature the β-whitelockite will be formed. Both formed are very tissue compatible and are used for bone substitute in a granular form or a solid block. We will consider the apatite form of calcium phosphate since it is regarded to be more closely related to the mineral phase of bone and teeth.