THE EFFECT OF DIFFERENT SINTERING TEMPERATURE TO THE PROPERTIES OF COW BONE HYDROXYAPATITE

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LIST OF ABBREVIATIONS

ACP	Amorphous Calcium Phosphates
ASTM	American Society for Testing and Materials
Al_2O_3	Aluminum Oxide
BSE	Backscattered
Bsp	Bone Sialoprotein
BSE	Bovine Spongiform Encephalopathy
BHA	Bovine Bone Apatite
$^oC/min$	Ramping Temperature
cm^3	Cubic Centimeter
CaO	Calcium Oxide
CO_3^{2-}	Carbonate
DTA/TG	Differential Thermal Analysis/ Thermal Gravimetric
DCP	Dicalcium Phosphate Anhydrous
DCPD	Dicalcium Phosphate Dihydrate
Dr.	Doctor
e.g.	For Example (exempli gratia)
et al.	And Others (et alia)
Fig.	Figure
FTIR	Fourier Transform Infrared Spectroscopy
Gpa	Giga Pascal
g/cm^3	Gram Per Cubic Centimeter
g/mol	Grams Per Mole
HIV	Human Immunodeficiency Virus (HIV)
IR	Infrared Spectroscopy

JCPDS	Joint Committee on Powder Diffraction Standards
K_{IC}	Fracture Toughness
kN	Kilo Newton
keV	Kilo Electron Volt
Mpa	Mega Pascal
mol/dm^{-3}	Moles per Decimeters Cube
MgO	Magnesium Oxide
ml	Milliliter
mm	Millimeter
M. Sc.	Master of Science
Mr.	Mister
MOSTI	Malaysian Ministry of Science, Technology & Innovation
MOR	Modulus of Rupture
NaOH	Sodium Hydroxide
N/mm^2	Newton per Square Millimeter
OH^-	Hydroxyl
Opn	Osteopontin
Ocn	Osteocalcin
ρ_{water}	1.0 g/cm^3
PEG	Polyethylene Glycol
PMMA	Polymethylmethacrylate
PO_4^{3-}	Phosphate
PTFE	Polytetrafluoroethylene
ppm	Parts Per Million
rpm	Revolutions Per Minute

SEM	Scanning Electron Microscope
SE	Secondary Electrons
SHMP	Sodium Hexametaphosphate
TTCP	Tetracalcium Phosphate
TCP	Tricalcium Phosphate
TiO_2	Titanium Oxide
s.w.t	Subhanahu Wa Ta'ala
SiC	Silicon Carbide
USM	Universiti Sains Malaysia
UHMWPE	Ultra-High Molecular-Weight Polyethylene
UTM	Universal Testing Machine
µm	Micrometer
UniMAP	Universiti Malaysia Perlis
Wt. %	Weight Percent
XRD	X-Ray Diffraction
XRF	X-Ray Fluorescence

LIST OF SYMBOLS

^{o}C	Temperature (Degree Celsius)
σ_f	Mega Pascal
%	Percentage
®	Original
β	Beta
F	Flourine
Cl	Chloride
Cd	Cadmium
Co	Cobalt
Cu	Copper
Fe	Iron
K	Potassium
Lo	Initial Length
Lf	Final Length
Na	Sodium (Natrium)
Ni	Nickel
Pb	Plumbum (Lead)
Zn	Zinc
As	Arsenic
Hg	Mercury
g	Gram
lo	Length
S_F	Percent of Firing Shrinkage (%)
D_f	Final Dimension of Sample After Firing

D_i	Initial Dimension of Sample
P	Percent of Porosity (%)
W_a	Samples Weighted in Air
W_b	Samples Weight in Water
W_c	Samples Weighted the Saturated Mass
F	Load of Fracture
L	Distance between Support Points
B	Width of the Sample
d	Thickness of the Sample

The Effect of Different Sintering Temperature to the Properties of Cow Bone Hydroxyapatite

ABSTRACT

Hydroxyapatite (HA) has been proved to be very compatible with the surrounding tissue of vertebrates since its chemical compositions similar to human bone and teeth. Natural HA has potential to be used as implant material. Main source of natural HA used are cow bones. Extraction of HA from cow bone is biologically safe and economic. In this study, effect of different sintering temperature to the properties of cow bone HA (CBHA) was carried out in two parts. First part is about characterization of raw materials. Temperature 800°C is chosen as calcination temperature. Fine CBHA is successfully produced via calcinations and milling process. From the result, particle size distribution for fine CBHA is 10.324µm. The phase obtained from XRD analysis is HA. Elemental analysis by XRF detects phosphorus (P) and calcium (Ca) with the Ca/P ratio of 1.67. Functional groups examined by FTIR detect hydroxyl (OH^-), carbonate (CO_3^{2-}) and phosphate (PO_4^{3-}). SEM shows non uniform distribution of particle. In the second part, effects of different sintering temperature (1150°C, 1200°C, 1250°C, and 1300°C) to the properties of CBHA were studied. The XRD results for CBHA correspond with standard HA for all temperature. Sintering temperature is inversely proportional with XRD peaks intensities. Shrinkage analysis shows that the higher percentage of shrinkage value is 47.65 % recorded at 1300°C. Whereas, density and porosity testing show that the lowest percentage of porosity value is 4.52 % at 1300°C and density reaches maximum value of 2.52 g/cm^3 for specimen at 1250°C and remains constant at 1300°. Mechanical properties observation through MOR testing shows that the highest value (35.14N/mm^2) obtained at 1300°. SEM analysis on the specimen sintered at 1300°C shows uniform grain size at the range 2 – 4 µm, with minimum amount of pore. As a conclusion the sintering temperature affect the physical, microstructure, phase and mechanical properties of CBHA.

Kesan Suhu Persinteran Berbeza Terhadap
Sifat-sifat Hidroksiapatit Tulang Lembu

ABSTRAK

Hidroksiapatit (HA) telah terbukti sangat bersesuaian dengan persekitaran tisu vertebrata memandangkan kandungan kimianya sama seperti tulang dan gigi manusia. HA semulajadi berpotensi untuk digunakan sebagai bahan implan. Sumber asal HA semulajadi yang digunakan adalah tulang lembu. Penghasilan HA dari tulang semulajadi secara biologinya selamat dan ekonomi. Dalam kajian ini, kesan suhu persinteran yang berbeza terhadap sifat-sifat HA tulang lembu (CBHA) telah dilaksanakan dalam dua bahagian. Bahagian pertama adalah berkenaan pencirian bahan mentah. Suhu 800°C dipilih sebagai suhu pengkalsinan. CBHA yang halus berjaya dihasilkan melalui proses pengkalsinan dan pengisaran. Daripada keputusan, taburan saiz partikal untuk CBHA yang halus ialah 10.324µm. Fasa yang didapati daripada analisa XRD ialah fasa HA. Analisa unsur melalui XRF mengesan fosforus (P) dan kalsium (Ca) dengan nisbah Ca/P ialah 1.67. Kumpulan berfungsi diperiksa dengan FTIR mengesan hidroksil (OH^-), karbonat ($CO^{2-}{}_3$) dan fosfat ($PO^{3-}{}_4$). SEM menunjukkan partikel dengan taburan yang tidak sekata. Dalam bahagian kedua, kesan suhu persinteran berbeza (1150°C, 1200°C, 1250°C dan 1300°C) terhadap sifat-sifat CBHA dikaji. XRD untuk CBHA semulajadi menunjukkan padanan dengan HA piawai untuk semua suhu. Suhu persinteran berkadar songsang dengan keamatan puncak. Nilai peratusan pengecutan tertinggi ialah 47.65% dicatat pada 1300°C. Nilai peratusan keliangan terendah ialah 4.52% pada 1300°C. Analisa pengecutan menunjukkan nilai peratusan pengecutan tertinggi ialah 47.65% dicatat pada 1300°C.Manakala ujian ketumpatan dan keliangan menunjukkan peratusan keliangan terendah ialah 4.52 % pada 1300°C dan ketumpatan mencapai nilai maksima pada 2.52 g/cm^3 untuk spedimen pada 1250°C dan tidak berubah sehingga 1300°. Pemerhatian sifat-sifat mekanikal melalui ujian MOR menunjukkan bahawa nilai tertinggi (35.14N/mm2) diperolehi pada 1300°. Analisis SEM pada spesimen yang disinter pada 1300°C menunjukkan saiz butiran yang seragam pada julat 2 - 4 µm, dengan jumlah liang minimum. Kesimpulannya suhu pensinteran menjejaskan sifat-sifat fizikal, mikrostruktur, fasa dan mekanikal daripada CBHA.

CHAPTER 1

INTRODUCTION

1.1 Background

The introduction of bioceramics and diversity of manufacturing techniques has broadened the applications that can be used in human body. The HA is a compound of a specific composition and defined crystal structure, $Ca_5(PO_4)_3(OH)$ (Shi, 2006). Albee in 1920 was first documented repairing bone defect with a reagent described as 'triple calcium phosphate compound' (Hench & Wilson, 1993). The HA has been clinically approved to be very compatible with the surrounding tissue of vertebrates since its chemical compositions similar human bone and teeth (Ramesh et al., 2007). HA have proven to posses hydrophilic surfaces and form intimate bonds with bone, unfortunately their brittle and poor tensile strength in nature limit the applications to be use (Billotte, 2003). Application of HA limited to non-bearing area such as inner ear bones or filler materials because poor mechanical properties, lack of reliability and brittle in nature (Zafer & Robert, 2007). HA can be either biological (natural) or as synthetic.

Synthetic HA can be produce via precipitation process, hydrolysis, hydrothermal. Precipitation process is the reaction of calcium nitrate with diammonium hydrogen phosphate. This process produces by product that can harm and pollution to the environment. In hydrolysis process, the reaction between phosphoric acid and calcium hydroxide produce by product water. Hydrolysis process requires highly controlled parameters such as pH and temperature.

Natural HA extract from natural source such as animal bones are different from synthetic HA in stoichiometry, composition, crystallinity, physical and mechanical properties. HA produced from natural bone have advantage that it inherits some properties of the origin bone such as its chemical composition and structure. Natural origin HA extracted from animal bones by their treatment with hot sodium hydroxide (NaOH) solution.

As an alternative, hydroxyapatite can be extract from natural resources. Efforts have been made by researchers on corals, cuttlefish bone, eggshells and pig bone to produce hydroxyapatite. According to Bahrololoom et al., 2009, bovine bone can be extract from bone ash. Merits of this extraction are removal of all organic part from bone and also prevent transmission of dangerous diseases. Cow bone is chosen as natural HA because in Malaysia it will be economic because it can be collect in large quantity at slaughter house and market. Cow bone as a waste material now can be used as biomaterial in implants and dental application.

Ceramics processing technology involved the study of principles, technology and processing the ceramics materials into desired fabricate products (James, 1995). Slip casting processes produced dense products of complex shape, size and structure. The advantages of slip casting is the product reproductions ability and complex geometries shape of product can be make with good materials homogeneity. Equipment and mould cost also economical such as low tooling and mould price. The disadvantages are limited low dimensional precision is needed with low production rate. The molds have low toughness and easily fracture so large molds inventory must be maintained to ensure constant production rate. The definition of sintering is the consolidation of the product during firing. Consolidation means particles have joined together into an aggregate that has strength (James, 1995). The basic types of sintering processes are solid-state sintering, sintering of glass particles, sintering of whiteware bodies (vitrification) and sintering of glazes and glassy thick films (James, 1995). Solid-state sintering processes are as sintering of a crystalline single phase materials and sintering of a single phase with a refractory dopant materials. The problem associated with sintering is the generation of thermal and residual stress fields due to low heat conductivity and high shrinkage of the HA. This will lead to the formation of micro or macro cracks in the specimen. As the solution, the temperature ramp usually not exceeds 5°C/min (Oleg & Igor, 2006). Sintering of HA results changes in crystal morphology, crystallinity and composition (Hench & Wilson, 1993). Mechanical strength and bioactivity of HA depend strongly upon its microstructure such as grain size and grain size distribution, porosity and its shape and distribution and material crystallinity (Sumit et al., 2007). Bone tissue grows well in the pores, increasing strength of the HA implants (Sopyan et al., 2007).

Firing process proceeds in three stages that are first reactions preliminary to sintering that include organic burnout and elimination of gaseous product of decomposition and oxidation proceeds with sintering as second stages and lastly cooling either thermal or chemical annealing (James, 1995). Sintering process of bone at 800°C will produce mainly HA and minor amount of CaO (Hench & Wilson, 1993). The sintering temperature for dense HA strongly depends on the powder properties and powder packing (Nithyanantham et al., 2002). Temperature between 1200°C and 1300°C is the optimum sintering temperature for maximum strength for biologically derived HA. Dense HA with high strength, hardness and high density will be produce when sintering at 1300°C (Goller & Oktar, 2002). The samples sintered above 1200°C exhibited very little porosity as compared to those sintered at 1200°C and below (Muralithran & Ramesh, 2000).

Various studies reportedly have been achieved on the improvement of powder processing and composition modification extensively. HA not strong as bone so, high sintering temperature resulted in better densification (Ramesh et al., 2007). Sintered density of natural HA was lower than artificial (synthetic) HA and detectable peaks from FTIR and XRD were identical to HA phase while natural HA features mostly HA phases and small amount of MgO (Kim et al., 2008).

Cow is defined as the mature female of any species of cattle particularly domesticated cattle. Bone generally composed of organic and inorganic components that also provide internal support for vertebrates. The organic part is contained mainly collagen and proteins, whereas the inorganic part is mainly HA with small percentage of other elements being incorporated in the structure such as carbonate, magnesium and sodium (Ooi et al., 2007). The formation of bone occurs in two basics processes that are osteoblasts (forming) and osteoclasts (resorbing). From microscopic level, woven and lamellar are two types of bone can be observed. Woven bone is the first bone to form in the embryo and can be recognize as disoriented arrangement of collagen fibers. Woven bone then gradually changes to lamellar bone except in a few places such as in tooth sockets. The structure of lamellar bones looks like highly organized and has collagen bundles oriented in the same direction. The structural organization of bone can be divided into two categories that are trabecular bone that appear as spongy and cancellous and the other one is known as cortical bone (compact) (Aziz, 2005).

1.2 Problem Statement

Research on extraction and production natural HA instead of synthetic HA that mostly researchers focus nowadays is important to find an alternative or as an option in clinical used for example in implant surgery. Animal bones as a source of raw materials are available in large quantities in nature.

From finding, researchers and scientists aims on producing HA from synthesizing method and result synthetic HA is produce. Synthetic HA not has enough biological properties same with that existed in human bone and teeth. Technique for processing and synthesize the synthetic HA also difficult and complicated. Dense HA is one of bioactive implant material which could attach directly by chemical bonding with the bone in body (Shi, 2006). HA synthesized from sources of natural origin such as bones of humans and animals can be safe provided if all safety precautions are fulfilled (Oktar, 2006). These project focus on characterization of cow bone as generally upon heating, powder characterization and study the effect of sintering temperature and soaking time on the porosity and density, strength and microstructures of dense natural HA.

In the previous study, calcinations and firing for several hours could overcome this problem since no protein can survive a high temperature up to 800°C (Hiroshi et al., 2007). HA produce also needed to formable into desired shape by economic technique without compromising its properties. This research aims to extract HA from natural sources that is cow bone since it has plenty of resources, high potential as raw materials, low cost of production, simple processing and similar structure to human bone. In addition, this research will use extraction method that biological safe to produce HA from cow bone.

1.3 Objectives of Research

1. To extract and characterize HA powder from cow bone.
2. To investigate the effect of different sintering temperature (1150^{o}C, 1200^{o}C, 1250^{o}C, 1300^{o}C) to the physical, microstructure, phase and mechanical properties of extracted cow bone HA.

1.4 Scope of Research

In this project, the effect of sintering temperature to the properties of CBHA was examined. These studies were dividing into 3 stages. In the first stage, cow bone was treated with sodium hydroxide (NaOH) at 100^{o}C for 48 hours. After discard unwanted materials, treated bone was dried at 150^{o}C in Memmert oven and calcined at 800^{o}C in Carbolite Muffle furnace. Calcined CBHA was milling in ball milling set fine CBHA powders. For raw powder examination, the particle size of powder was investigated by Malvern Mastersizer analyzer machine.

The samples were prepared by mixed CBHA powder with calgon (sodium hexametaphosphate) for 16 hours. Then mix powder blend with polyethylene glycol (PEG) for 4 hours. PEG is use as a binder in pressing. The mixture was compacted 50 MPa for 1 minute using uniaxial hydraulic press machine. The compacted specimen were sintered by Carbolite Muffle furnace at 1150^{o}C, 1200^{o}C, 1250^{o}C and 1300^{o}C at 3 hours sintering temperature with 5^{o}C/min heating rate.

At the final stage of study, several examinations and testing were carried out. The samples were measured firing shrinkage. Scanning electron microscope (SEM) was observed specimen morphology. Functional group information was examined by Fourier transform infra red spectroscopy (FTIR) by Perkin Elmer (*Spectrum RX1*). XRF was used for elemental analysis. Strength of sintered samples is measure by Instron Strength Testing Machine with a three-point bending geometry based on ASTM C1161. The density and porosity of the samples were determined based on Archimedes principle. Phase transformation and thermal behavior of sample were study using X-Ray Diffraction Shimadzu XR 2000 and TGA model *Pyris Diamond*.

CHAPTER 2

LITERATURE

2.1 Biomaterials

The term biomaterials can be defined as a materials used to replace part of a living system or to function in intimate contact with living tissue (Joon, 2000). Biomaterials also refer to human made materials used to construct prosthetic or other medical devices in implantation process. The use in medical field are such as replacement segment of bone with similar structure of titanium alloy having better strength despite origin of metallic materials lack of ability to adapt with bone (Scott, 2005).

Biomaterials usually are in form of an implant components apply in human body to replace damaged or diseased body part. Noticeable character such as compatible with body tissue, free from toxic substances and not cause any adverse biological reactions must be features in all biomaterials characteristic (Shi, 2006). Biomaterial is not biological materials, such as bone that produced from biological system. A special characteristics commonly features to bioactive materials is can bonding with human bone without fibrous tissue at the interface. Performance of reliably biomaterials depends on materials properties, design and biocompatibility, surgical technique, health condition and other perspective that should be done in good manner (Joseph, 2000). Biomaterials also can be understand as a human made materials used to construct prosthetic or other medical devices for implantation in human body (Scott, 2005). Table