



**UNIVERSITI PUTRA MALAYSIA**

***PHYSICOCHEMICAL PROPERTIES AND BIOACTIVITY OF  
HYDROXYAPATITE/ZIRCONIA BIOCOMPOSITE PREPARED  
BY BALL MILLING***

**FATEMEH MOHAMMADDOOST**

**FK 2016 87**



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BY BALL MILLING**

By

**FATEMEH MOHAMMADDOOST**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfilment of the Requirements for the Degree of Master of Science**

**April 2016**

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## DEDICATION

This thesis work is dedicated to my father Morteza Mohammaddoost and my mother Farahnaz Malayeri, who have been a constant source of support and encouragement during the challenges of graduation and life. This work is also dedicated to my lovely brother Ali Mohammaddoost and my beloved Alaa Abdulrahman Tabaan who love me unconditionally and whose good examples have taught me to work hard for the things that I aspire to achieve. A token of appreciation to everyone, who has worked for freedom and peace whole the world.



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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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**FATEMEH MOHAMADDOOST**

**April 2016**

**Chairman : Hamdan Mohamed Yusoff, PhD**  
**Faculty : Engineering**

The superior biocompatibility and bioactivity of hydroxyapatite (HA) ceramic has attracted much attention as a substitute material in bone grafting. However, the mechanical properties of HA are low in comparison with cortical bone. Therefore, Zirconia ( $ZrO_2$ ) is a well-known material, which is bioinert material. The effects of Zirconium oxide ( $ZrO_2$ ) additive on the microstructure and physical properties of hydroxyapatite (HA) as well as on the bioactivity were investigated in this study. The HA- $ZrO_2$  powder was derived from natural bovine bone by a high energy milling technique with specified sintering temperature. In the present work, HA- $ZrO_2$  bio ceramic were produced at various sintering conditions (1150, 1200, 1250 and 1300 °C) with different  $ZrO_2$  concentrations (0.0, 0.2, 0.4 and 0.8 wt%) in two different milling times (30 min and 1 h), hence the effects of the amount of  $ZrO_2$  in the biocomposite on the structure and mechanical properties were investigated. All samples showed HA as a major phase and beta-tricalcium phosphate ( $\beta$ -TCP) and alpha-tricalcium phosphate ( $\alpha$ -TCP) phases as a minor phase, which also showed tetragonal zirconia (t- $ZrO_2$ ) phase. The XRD results showed that the decomposition of HA (amount of  $\beta$ -TCP and  $\alpha$ -TCP phases) increased with the increasing amount of additive ( $ZrO_2$ ). Furthermore, the additive inhibited grain growth as a result of a decrease in grain size (as shown in the SEM images). The density was determined by Archimedes method and the results showed that the highest density ( $2.97 \text{ g/cm}^3$ ) was achieved for a biocomposite that sintered at 1250 °C containing 0.2 wt% of  $ZrO_2$ , for 1 h milling time, which, is consistent with microhardness data. The highest value of microhardness was 286 HV, which was observed for the same sample as the highest density value. Besides that, the density and microhardness values were increased by increasing the milling time from 30 min to 1 h. This could probably be due to the increase of t- $ZrO_2$  by increasing the milling time, which was observed in XRD results. In conclusion, the mechanical properties increased by increasing the milling time from 30 min to 1 h. The in-vitro method was used to test the bioactivity of the biocomposite. For instance, bioactivity was studied by soaking the samples in the SBF solution for two different periods of time, namely 7 and 15 days, followed by the SEM, EDX analysis as well as XRD. The SEM results showed the apatite on the

surface of HA-ZrO<sub>2</sub> biocomposite on a 7 day growth and when the immersion time increased to 15 days, the growth of apatite on the surface increased more. Other than that, the EDX showed that the covered layer on the surface was P and Ca as well as O. The XRD results showed that the soaked HA-ZrO<sub>2</sub> biocomposite composed HA and ZrO<sub>2</sub> and no other phases were detected. Also, ZrO<sub>2</sub> reduced the dissolution rate of the biocomposite in the SBF. From the findings, we concluded that the addition of ZrO<sub>2</sub> to HA increased the mechanical properties and bioactivity of the biocomposite.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**FIZIKO- KIMIA PROPERTIES DAN BIOAKTIVITI DARIPADA  
HYDROXAPATITE / ZIRCONIA BIOKOMPOSIT DISEDIAKAN OLEH  
HIGH ENERGY BALL MILLING TECHNIQUE**

Oleh

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**April 2016**

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Keunggulan biokeserasian dan bioaktiviti daripada hydroxyapatite (HA) seramik telah menarik perhatian pelbagai pihak untuk digunakan sebagai bahan gantikan dalam cantuman tulang. Walau bagaimanapun, sifat-sifat mekanik HA adalah rendah berbanding dengan tulang kortikal. Oleh itu, zirkonia ( $ZrO_2$ ) sejenis bahan yang terkenal, yang juga merupakan bahan bioinert. Kesan bahan tambahan zirkonium oksida ( $ZrO_2$ ) kepada mikrostruktur dan sifat fizikal hydroxyapatite (HA) dan juga bioaktiviti telah disiasat dalam kajian ini. Serbuk HA- $ZrO_2$  yang berasal dari tulang lembu dihasilkan menggunakan teknik pengisaran bebola bertenaga tinggi pada suhu pensinteran yang ditetapkan. Dalam kajian ini, HA- $ZrO_2$  bioseramik dihasilkan pada pelbagai suhu pensinteran (1150, 1200, 1250 dan 1300 °C) dan kandungan  $ZrO_2$  yang berbeza (0.0, 0.2, 0.4 dan 0.8 wt%) dalam dua masa pengisaran yang berbeza (30 min dan 1h), maka kesan daripada jumlah  $ZrO_2$  dalam komposit kepada struktur dan sifat-sifat mekanik telah disiasat. Semua sampel menunjukkan HA sebagai fasa utama dan beta- trikalsium fosfat ( $\beta$ -TCP) dan alpha- trikalsium fosfat ( $\alpha$ -TCP) fasa sebagai fasa kecil, yang juga menunjukkan fasa zirkonia tetragonal (t-  $ZrO_2$ ). Keputusan XRD menunjukkan penguraian HA (jumlah fasa  $\beta$ -TCP dan  $\alpha$ -TCP) meningkat dengan peningkatan kepekatan bahan tambahan ( $ZrO_2$ ). Tambahan pula, bahan tambahan menghalang pertumbuhan butiran akibat penurunan dalam saiz butiran (seperti yang ditunjukkan dalam imej-imej SEM). Ketumpatan pula ditentukan dengan kaedah Archimedes dan keputusan menunjukkan bahawa ketumpatan tertinggi (2.97 g/cm<sup>3</sup>) telah dicapai oleh komposit yang disinter pada 1250 °C dan mengandungi 0.2 wt%  $ZrO_2$ , bagi masa pengilangan 1 h, selari dengan data kekuatan mikro. Nilai tertinggi kekuatan-mikro adalah 286 HV, dan sampel yang sama mencatatkan nilai ketumpatan tertinggi. Selain itu, ketumpatan dan kekuatan mikro nilai-nilai telah meningkat dengan penambahan masa pengisaran dari 30 minit hingga 1 jam. Ini dapat mungkin disebabkan oleh peningkatan sebanyak t- $ZrO_2$  dengan meningkatkan masa pengisaran, yang diperhatikan dalam keputusan XRD. Kesimpulannya, sifat-sifat mekanikal meningkat dengan penambahan masa pengisaran dari 30 minit kepada 1 jam. Kaedah in-vitro juga telah digunakan untuk menguji sifat bioaktiviti komposit. Sebagai contoh, aktiviti biologi telah dikaji

dengan merendam sampel dalam larutan SBF selama dua tempoh masa yang berbeza, iaitu 7 dan 15 hari, diikuti dengan analisis SEM, EDX serta XRD. Keputusan SEM menunjukkan pertumbuhan apatite pada permukaan HA-ZrO<sub>2</sub> biokomposit selepas direndam 7 hari dan apabila masa rendaman meningkat kepada 15 hari, pertumbuhan apatite di permukaan meningkat. Selain daripada itu, EDX menunjukkan bahawa lapisan yang melitupi permukaan komposit adalah P dan Ca serta O. XRD juga menunjukkan selepas direndam, HA-ZrO<sub>2</sub> biokomposit terdiri HA dan ZrO<sub>2</sub> dan tidak ada fasa lain dikesan. Juga, ZrO<sub>2</sub> mengurangkan kadar peleraian komposit dalam SBF. Dari hasil kajian, kita membuat kesimpulan bahawa penambahan ZrO<sub>2</sub> ke dalam HA akan meningkatkan sifat mekanik dan bioaktiviti komposit.





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I certify that a Thesis Examination Committee has met on 15 April 2016 to conduct the final examination of Fatemeh Mohammad Doost on her thesis entitled "Physico-Chemical Properties and Bioactivity of Hydroxyapatite/Zirconia Biocomposite Prepared by Ball Milling" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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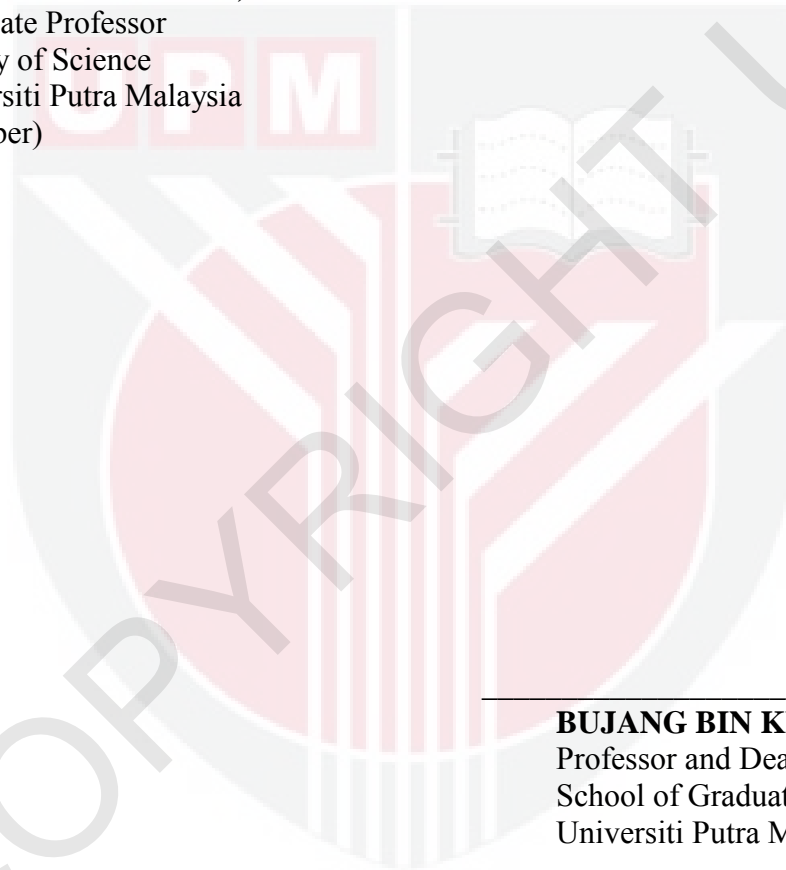
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## LIST OF ABBREVIATIONS

$(\text{CH}_2\text{OH})_3\text{CNH}_2$	Tris (Hydroxymethyl) Aminomethane
$\text{CaCl}_2$	Calcium Chloride
HA	Hydroxyapatite
HCA	Hydroxy-Carbonated Apatite
HCl	Hydrochloric Acid
JCPDS	Joint Committee On Powder Diffraction Standards
KCl	Potassium Chloride
M	Mess
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	Magnesium Chloride Hexahydrate
Mpa	Mega Pascal
N	Newton
$\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$	Di-Sodium Hydrogen Phosphate Dehydrate
$\text{Na}_2\text{SO}_4$	Sodium Sulphate Anhydrous
NaCl	Sodium Chloride
$\text{NaHCO}_3$	Sodium Bicarbonate
SBF	Simulated Body Fluid
SEM	Scanning Electron Microscope
TCP	Tri Calcium Phosphate
V	Volume
XRD	X-Ray Diffraction,
Zr	Zirconium
$\text{ZrO}_2$	Zirconia (Zirconium oxide)
P	Density

# CHAPTER 1

## INTRODUCTION

### 1.1 Background

All humans face aging, which may cause damage or loss of soft or hard tissues in their biological system as well as accidents which can damage the biological system. For instance, bone is naturally constructed from an inorganic-organic biocomposite consisting mainly of collagen proteins and hydroxyapatite (Qu, 2007).

Materials used as bone graft have been classified in different categories, The first group is natural materials (auto-graft, allograft and xeno-graft) and the second group is synthesised materials (metals, ceramics and polymers) (Janas and TenHuisen, 2003; Kulkarni, 2013). Natural material, like auto-grafts, takes the bone from another site of patient to be used for the new part is the most preferred bone graft. The allograft materials are materials which need a donor, which means the bone implant is harvested from other people's bone skeleton, however, this is very risky and more painful (Qu, 2007).

Synthesised materials constitute another large family of bone graft substitutes. Because of their similarity to the native auto-grafts, it has been attractive to produce it as an artificial bone used in medical applications. These biological materials are considered to be implanted in the human body to perform specific biological functions by replacing a variety of tissues such as bone, cartilage and tendons. Metals, polymer, ceramic and their biocomposite are traditionally used as hard tissue materials. The earliest biomaterial that used for hard tissue replacement are metals and polymers (Kulkarni et al., 2013).

Ceramic materials are increasingly used for repair and reconstruction of skeletal diseases and disorders for recent decades. These bioceramic materials, particularly calcium phosphate-based materials, are being preferred due to biocompatibility, better bone and tissue bonding abilities, and the similarity with the inorganic components of human bone.

### 1.2 Problem statement

Each year, about two million people worldwide undergo some form of bone rafting procedure to repair handicap either due to disease or injury. Consequently, the bone graft market has been predicted to be worth around one billion US dollars annually (Hore, 2011).

The main goal of making bioceramic materials is to improve the properties of the material use as a medical application. Due to special properties and high similarity to cortical bone, bio-ceramic is the best candidate to replace the hard and soft tissues among all the biomaterials (Wang, 2003; Vonder et al., 2010). The main advantages of synthetic HA are biocompatibility and good osteoconductive (Legeros, 1993; Vonder et al., 2010).

The preparation methods of synthetic HA can be classified into four groups namely dry methods (mechanochemical synthesis, solid state method), wet methods (sol-gel synthesis, hydrothermal reaction, micro emulsion syntheses), high-temperature processes (combination procedures and pyrolysis method), and synthesis from biogenic sources (fish bone, bovine bone, eggshell) (Sadat-Shojai et al., 2013). Generally, the costs of raw materials for the preparation of HA, especially for the chemical route are quite high. The quality of most of the product, which synthesised from the chemical route are not good, while the reaction is very risky and the chance to get high yield of product is low. For example, solid state reaction route is one of the easiest techniques to understand but it is difficult to adjust the starting purity of raw materials. Furthermore, the route usually produces inhomogeneous nonstoichiometric HA which require high temperature for HA formation (Riman et al., 2002). Another good candidate with conventional calcination can be the HA powder, which is produced from natural resources (Herliansyah et al., 2007; Ooi et al., 2007). Other than that, the process of producing HA from natural sources is simple and practical with low cost and at the same time is environmentally used (Ruksudjarit et al., 2008; Kantana et al., 2013). Natural HA bio ceramic has been reported to be extracted by normal calcinations of some bio-waste, such as fish bones (Ozawa and Suzuki, 2002), marine crustaceans (Sampath Kumar et al., 2000), eggshells (Dupoirieux, 1999; Dupoirieux et al., 2001) and ostrich egg shell (Rivera et al., 1999) and bovine bone (Joschek et al., 2000; Barakat et al., 2009; Younesi et al., 2010).

Among of all natural sources bovine bone has been selected (Joschek et al., 2000; Barakat et al., 2009; Younesi et al., 2010). The recent research shows that current global average meat consumption is 100 g per person per day and this much consumption leads to produce more waste (Michael et al., 2007). Furthermore, one of the important issues in the industrial process is the minimization of waste and recycling it into useful products. The bovine bone can be considered as one of the waste that can be collected from the local slaughterhouses. Hence, developing a simple method to convert these bovine bones into HA is essential from the viewpoint of environmental science and engineering. Therefore, in this work used bovine bone as source of HA.

Moreover, in most application conditions, single phase ceramic cannot achieve the application requirements, as it has either low bioactivity or low mechanical strength. Therefore, the mechanical properties of HA are low in comparison with cortical bone (Egusa et al., 2008). Besides that, considerable improvement in the hardness and density of HA without compromising biocompatibility was the main aim of many researches during past years (Mittal et al., 2013). In order to increase the mechanical

properties of the HA, an incorporation of resistant oxide phases like carbon, titanium, cobalt-chromium alloys and zirconia is required to optimise the biocompatibility and improve the mechanical properties of the biocomposite (Kantana et al., 2013). In this study zirconia ( $ZrO_2$ ) is used to improve mechanical properties (density and microhardness) as well as bioactivity of HA (Curran et al., 2009; Kantana et al., 2013).

Zirconia ( $ZrO_2$ ) based ceramics have been gaining much attention in recent years due to their relatively high fracture toughness as compared with other ceramics (Curran et al., 2010). Furthermore,  $ZrO_2$  is a bio inert ceramics with unique stress-induced ability for biomaterial , which has been used in some hip and knee replacement devices for having good biocompatibility as well as excellent wear properties (Uchida et al., 2001). Thus, the HA- $ZrO_2$  biocomposite materials may be an excellent biocomposite for orthopedic implantation (Quan et al., 2013). Other than that,  $ZrO_2$  is used as an additive to enhance the mechanical and physical properties of HA such as density and hardness.

$ZrO_2$  is a good candidate for increasing mechanical properties due to nontoxic properties at stable body fluid, (Chevalier, 2006). It's Incredibly inert (Kutty and Singh, 2001), and being a material with high resistance to crack propagation (Kantana et al., 2013). Table 1.1 shows the recent research of HA- $ZrO_2$  biocomposite.

**Table1.1: Researches of HA-ZrO<sub>2</sub> biocomposite**

Method	ZrO <sub>2</sub> %	Finding	References
Pressureless sintering	20-40 (wt)	The hardness and wear resistance increased with the increase of the volume percentage of zirconia	(Wang et al., 2005)
Cold press	3-8 (wt)	Addition of zirconia caused increased decomposition of the HA at 1100 °C and 1300 °C, to TCP. The porosity of the sintered biocomposite increased with the increase in zirconia concentration.	(Evis, 2007)
Ultrasonic	0.0- 1.0 (vol)	The grain size of HA in HA-ZrO <sub>2</sub> biocomposite was much smaller than that in pure HA ceramic, ordinary	(Kantana et al., 2013).
spark plasma sintering	10-40 (wt)	HA/Y-TZP biocomposite could be densified at 1200 °C, the average HA grain size in the biocomposite layers was reduced.	(Guo et al., 2004)
Hydrothermal	10 (mol)	HA, t-ZrO <sub>2</sub> , TCP and CaZrO <sub>3</sub> exist in the HA-ZrO <sub>2</sub> biocomposite and the analysis of the biocomposite showed the formation of β-tricalcium phosphate due to the decomposition of HA.	(Curran et al., 2009)
	0-5 (wt)	ZrO <sub>2</sub> up to 3 wt% increased apparent density to 92%.	(Sivakumar and Manjubala, 2001)
solid state reaction	10-30 (wt)	High-temperature solid state reaction route resulted in more dense and more thermally stable HA-ZrO <sub>2</sub> biocomposite.	(Rao., 2002)
Uniaxial pressing sintering	20-80 (wt)	20 wt% showed the higher mechanical properties.	(Lim et al., 2014)
ball milling technique (normal milling)	5 and 10 (wt)	The incorporation of zirconia did not result in very strong BHA-biocomposite likely because of the big gap between the sintering temperatures of BHA and these oxides.	(Oktar et al., 2007)



A comprehensive review on HA and HA-ZrO<sub>2</sub> biocomposite revealed that high energy milling technique was used for biocomposite powder preparation, which is more likely to produce a powder with good mechanical properties and at the same time is low cost and customized the energy. We can mention that the other studies also focused on the other methods of processing of HA-ZrO<sub>2</sub> biocomposite such as solid state route (Rao., 2002), due to the sheer simplicity of the process. However, this process resulted in a less dense biocomposite with high decomposition of HA as well as lower amount of tetragonal Zirconia (t-ZrO<sub>2</sub>) and formation of TCP, tetra calcium phosphate, Calcium zirconated (CaZrO<sub>3</sub>). It is important to know that HA has affinity towards ZrO<sub>2</sub>, whereby this reaction in the HA-ZrO<sub>2</sub> leads to the decomposition of HA, and the loss of ZrO<sub>2</sub> in the biocomposite. The presence of large amount of ZrO<sub>2</sub> provides barrier to the densification process and speeds up the decomposition of HA to TCP along with the conversion of TZP to CaZrO<sub>3</sub> (Rao and Kannan, 2002; Evis, 2007). All these techniques which mentioned in Table 1.1 are aimed at reducing the particle size and sintering temperature of preparation the compound even though they are more involved and complicated in approach than the synthesis of HA-ZrO<sub>2</sub> biocomposite from bovine bone by high energy milling technique which derived in this study. In addition, most of the investigations on HA-ZrO<sub>2</sub> biocomposite contained high concentrate of ZrO<sub>2</sub>. Hence, there are not much research on the densification behaviour and mechanical properties of HA-ZrO<sub>2</sub> biocomposite containing low concentration of ZrO<sub>2</sub>. It is possible due to the apprehension that low percentage of fraction ZrO<sub>2</sub> may not enhance properties as desired and another reason may be due to difficulty of uniform dispersion of low percentage of zirconia in HA matrix. But since the ZrO<sub>2</sub> is lower it will cause less interaction and will help to retain both HA and ZrO<sub>2</sub> phases. To our knowledge this study of the synthesis of HA-ZrO<sub>2</sub> biocomposite from bovine bone with low concentrate of ZrO<sub>2</sub> by high energy milling technique has not been reported yet. Thus the reduced cost of first material by using bovine bone as source of HA reducing the energy by less milling time as compared to other researches sintering at lower temperatures with good results terms of mechanical properties and bioactivity of the biocomposite have investigated in this study.

### 1.3 Aims and Objectives

This project aims to develop HA-ZrO<sub>2</sub> biocomposite with high mechanical properties and superior bioactivity as a bone replacement material in medical applications. In this project, bioceramic HA-ZrO<sub>2</sub> will be prepared by high energy milling technique and sintering, the biocomposite material will be synthesised from biogenic sources (bovine bone) and the effects of various parameters such as temperature, different concentration of additive as well as milling time will be discussed.

The objectives of this project are:

1. To investigate the effects of sintering temperatures, milling time and ZrO<sub>2</sub> content on the structure and mechanical properties of the HA-ZrO<sub>2</sub> biocomposite.
2. To perform bioactivity test on the HA-ZrO<sub>2</sub> biocomposite.

#### 1.4 Scope of study

The scope of our study is to synthesise the HA-ZrO<sub>2</sub> biocomposite from bovine bone with different concentrations of ZrO<sub>2</sub> (0.2 to 0.8 wt%) by applying high energy milling technique in two different milling times (30 min and 1 h milling time) at different sintering temperatures (1150-1300 °C). This is done to make use of bioceramic in the medical applications, specifically for knee replacement. This synthesis has very low cost and is environmental friendly. Improving the mechanical properties of HA will allow HA to be used as an implant in the human body. Thus, ZrO<sub>2</sub> was chosen to improve the mechanical properties of HA for being bioinert and biocompatibility. Besides that, the phase's formation of the sintered samples was analysed by X-ray diffraction technique (XRD). The mechanical property and hardness value were investigated using a Vickers microhardness tester and density by Archimedes method. The microstructural investigation of the samples was also performed using a scanning electron microscope (SEM).

For the bioactivity test, the samples were immersed in to a simulated body fluid (SBF) for 7 and 15 days. After immersing in SBF, surface samples were studied by SEM and EDS techniques and the phase's formation after immersing in SBF by XRD as well as weighing the samples before and after immersing in SBF solution.

#### 1.5 Importance of study

Nowadays, our living condition has improved, and many people pay more attention to medical care and rehabilitation. For instance, there are more than million surgeries in the world, due to the injuries on the human's hard tissue system as well as aging. Bone grafts are second to blood transfusions on the list of transplanted materials. In order to get a successful bone replacement surgery, it is important for the bone graft material to be good in terms of the mechanical and biological properties. One of the serious health conditions in the world is bone and joint problem-diseases that millions of people are suffering from (Ngoi and Sreejith, 2000). The cost of preparing the HA is very cheap as it uses natural sources. Producing HA with old methods requires several chemical actions as they are complicated and expensive, which make it uneconomical and too risky. However, a good result can be obtained with low risk by using very simple thermal method synthesis from biogenic sources.

A report from London's institute of biomaterial indicated that the market of for biomaterial per year is around 12 billion dollars and this market is growing in the range of 7-12 percent annually (Joschek et al., 2000). Besides that, the global orthopedic market is estimated by Espicom to have been worth approximately 37.1 billion US dollars in 2008. Excluding arthroscopy and 'other' segments (operating theatre equipment and supplies), the market totaled 29 billion US dollars, having grown by 10.7 percent over the year (Venugopal et al., 2010). The valorisation of waste by products to obtain valuable compounds is a method that has become more popular in recent years. The main reason for increasing the large amount of waste is producing more, which need to be disposed. Thus, there are a huge potential to use them as a source of the compounds (Piccirillo et al., 2013).

HA is the main component of animal and fish bones (Mandal et al., 2010). It accounts for between 60 to 70% of their weight, depending on both the animal and on the type of bones considered, in which the remaining part of the bone consists of organic molecules, mainly collagen. For this reason, the bone can be used as a raw material to produce commercial HA (Piccirillo et al., 2013).



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