DESIGN AND DEVELOPEMENT OF HYDROXYAPATITE/ZIRCONIA (HA/ZrO_2) SCAFFOLD FOR GUIDED BONE REGENERATION

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For the love one and his prophet.
Specially dedicated to my father, Mamat bin Muda, and my mother,
Laili binti Mukhtar and to all my friends.

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ABSTRACT

Highly porous scaffold made of bioceramics such as hydroxyapatite (HA) have been widely investigated for guided bone regeneration, however their inferior mechanical strength limits their clinical use to non load bearing applications. In this study we have synthesised HA/ zirconia (ZrO_2) mixtures (5, 10, and 20 wt%) using microwave assisted wet precipitation method. Phase composition and particle morphology of the mixture was studied using X-Ray Diffraction (XRD) and Scanning Electron Microscopy (SEM) respectively. Presence of ZrO_2 in mixtures was confirmed by the presence of peak at 30.22° in XRD, which increased in intensity as concentration of ZrO_2 increased in the mixture. Porous structures were generated by using polymeric sponge burn off method. Solid loading of sponge was optimised to achieve good degree of porosity, pore size and pore interconnectivity. SEM analysis of scaffolds revealed good pore interconnectivity and pore size greater than 200 microns. Scaffolds were dipped in solution of poly-L-lactic acid and HA/ZrO_2 prior to the compressive strength measurements. The compressive strength of scaffold increased from 1.73 to 2.47 MPa when ZrO_2 content increased from 5% to 20% for 20 wt% solid loading. For 30 wt% solid loading, compressive strength increased from 1.83 to 2.63 MPa as concentration of ZrO_2 increased.

ABSTRAK

Bahan perancah yang mempunyai keporosan yang tinggi yang diperbuat daripada bioseramik seperti hidroksiapatit (HA) telah banyak dikaji untuk memandu pertumbuhan semula tulang, namun kekuatan mekanikalnya lebih rendah mengehadkan penggunaan klinikalnya untuk aplikasi bukan galas beban. Dalam kajian ini, kami telah sintesis mencampurkan HA/zirconia (ZrO_2) dengan nisbah (5, 10 dan 20 wt%) dan menggunakan kaedah pemendakan basah dengan bantuan gelombang mikro. Komposisi fasa dan morfologi zarah sebatian telah dikaji masing-masing dengan menggunakan pembelauan X-Ray (XRD) dan pengimbas mikroskopi electron (SEM). Kehadiran ZrO_2 di dalam sebatian ditentukan oleh kehadiran puncak pada 30.22° didalam XRD, dimana keamatan meningkat dengan penambahan kepekatan ZrO_2 didalam sebatian. Struktur berliang telah dihasilkan dengan menggunakan kaedah membakar span polimer. Memuatkan pepejal dalam span dioptimumkan untuk mencapai darjah keporosan yang bagus, saiz liang dan liang yang saling bersambung. Analisis SEM bahan perancah menunjukkan rongga yang saling bersambung dan saiz rongga yang lebih besar daripada 200 mikro. Bahan perancah direndam kedalam asid larutan poli-L-laktik dan HA/ZrO_2 untuk meningkatakan ukuran kekuatan kemampatan. Kekuatan mampatan bahan perancah meningkat daripada 1.73 hingga 2.47 MPa apabila kandungan ZrO_2 meningkat daripada 5% kepada 20% bagi 20 wt% kemasukan pepejal. Bagi 30 wt% kemasukan pepejal, kekuatan mampatan meningkat daripada 1.83 hingga 2.63 MPa dengan peningkatan kepekatan ZrO_2.

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LIST OF ABBREVIATIONS

HA	-	Hydroxyapatite
t-ZrO_2	-	Tetragonal zirconia
B-TCP	-	Beta tricalcium phosphate
ZO	-	Zirconia
FWHM	-	Full width at half maximum
SEM	-	Scanning electron microscopy
XRD	-	X-ray diffraction
PLLA	-	Poly (L) lactic acid
PVA	-	Polyvinyl alcohol
TCP	-	Tricalcium phosphate
PSZ	-	Partially stable zirconia
WT	-	Porous calcium silicates
PBS	-	Phosphate buffered saline

LIST OF SYMBOLS

Nm	-	Nano meter
Λ	-	Wavelength
E	-	Degree of porosity
V	-	Volume
MPa	-	Mega Pasca
%	-	Percentage
GPa	-	Giga Pasca
°C	-	Degree of celcius
3D	-	Three dimension
~	-	Approximately
KPa	-	Kilo pasca
mg/cm^3	-	Mili gram per centimeter cube
g/cm^3	-	Gram per centimeter cube
W	-	Watt
Min	-	Minute
KN	-	Kilo Newton
D	-	Density
E	-	Degree of porosity

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Human beings are likely to face serious problems caused by diseases, accidents and aging during their lifetime. In the past physicians had very limited material choices on solving these medical problems with the removal of the ill functioning parts. Advances in science and technology of medical materials today significantly altered the choices of physicians for the solution of the implantation problems (Hench, 2002).

Biomaterials are defined as materials of natural or man-made origin that are used directly, as a supplement, or to replace the function of living tissues of human body. Two important criteria which biomaterial must fulfill are biocompatibility and biofunctionality. According to a report published in 1995 by the Institute of Materials London, the estimated world market for biomaterials was around $12 billion per year (Ramakrishna, 2001), which gives an idea of the size of biomaterials industry.

Biomaterials have much more importance than the economical aspect because they have vital importance for human life in many cases. The skeletal system provides support and gives shape to the body and provides a network for soft tissues to attach to. The most common problem associated with hard tissues is bone fractures, in addition to other various problems which also require medical attention and use of biomedical materials. The developments in artificial bone area seem to solve most of the problems related to hard tissue. On the other hand, artificial bones themselves may cause other problems as in some cases they do not have adequate mechanical properties to replace the original hard tissue. Ceramics, polymers, metals and composite materials have been developed to be used for bone repair and replacement purposes. Polymers have very low mechanical strength compared to bone, metals have superior mechanical properties but they are prone to corrosion in a biological environment and despite their other desired properties such as wear resistance, biocompatibility and hardness, ceramics are brittle and have low fracture toughness. However, combining the properties of these materials through the synthesis of composite materials seems to be good approach to overcome the shortcomings of their individual properties.

Bone consists of 69 wt% calcium phosphate (mainly hydroxyapatite), 21% collagen, 9% water and 1% other constituents (Park, 1992). It has a composite nature which is composed of mainly ceramic (hydroxyapatite) and polymer (collagen), with a complex hierarchical microstructure impossible to imitate which gives most of the superior mechanical properties to bone. There are many researches done for bone substitute composite materials composed of mainly hydroxyapatite and a polymer. Hydroxyapatite has very good properties such as bioactivity, biocompatibility, non-toxicity and osteoconductivity but also has low toughness.

Scaffolds play an important role in tissue engineering for bone regeneration because it act as template for cell interaction and formation of bone extracellular matrix that provide structural support to the formation of tissue. Porosity is important criteria in making scaffold. Porosity is defined as percentage of void space in solid. Porosity is important for the tissue formation process because it allows cell migration. Porous surface also plays an important role because it can give or improve mechanical interlocking between implant biomaterial and surrounding hard tissue and also can give a good mechanical stability. There are many techniques that have been used to make scaffold such as salt leaching, gas forming or phase separation. In order to get a good scaffold for bone regeneration purposes the minimum pore size should be 100 µm (Bhaskar, 1971).

1.2 Hydroxyapatite (HA)

Hydroxyapatite (HA), is a naturally occurring mineral with the formula $Ca_5(PO_4)_3(OH)$, but is usually written $Ca_{10}(PO_4)_6(OH)_2$ and it is a member of calcium phosphate family. Nowadays, calcium phosphate (CP) family has been used by surgeons and clinicians because it offers an array of synthetic bone replacement materials (Silva *et al.*, 2009). Moreover, up to 50% of bone is made up of a modified form of the inorganic mineral HA (known as bone mineral) (Park, 1992). For this reason, many research studies have used HA in tissue engineering. HA is a bioactive ceramic that has been widely used in bone repair and also as coating material for metallic prostheses to improve biological properties. From the point of view of biocompatibility, HA seems to be the most suitable ceramic material for the hard tissue replacement since it is very similar in composition to the main mineral constituent of bones. HA Ceramics are known to show no toxic effects and at the same time show excellent biocompatibility with hard tissues, skin and muscle tissues. Moreover, it can also bond directly to bone (Hench, and Wilson, 1993). In addition, HA is the most stable calcium phosphate ceramic under physiological conditions. While non-stoichiometric HA ($Ca/P \neq 1.67$) it can make thermally unstable that giving rise other CP Phase at elevated temperature resulting in poorly dense compact. Therefore it is important to ensure that the Ca/P stoichiometric ratio of synthetic HA for load bearing application is kept close to 1.67 (Bi *et al.*, 2009). Many research studies have used different sintering techniques to improve the mechanical properties of HA. In addition, mechanical properties of HA have been previously enhanced by the incorporation of different resistant oxides into HA (Kasuga *et al.*, 1993, Takaqi *et al.*, 1992). In this study, we have used partially stabilized zirconia to improve mechanical properties of HA scaffolds. There are many approaches that been used to synthesis hydroxyapatite e.g. sol-gel, hydrothermal processing, microwave method, wet precipitation and emulsion system.

1.2.1 Properties of Hydroxyapatite

Some of the mechanical properties of synthetic calcium phosphate (hydroxyapatite) are listed in Table 1.1.

Table 1.1: Properties of synthetic calcium phosphate (hydroxyapatite) (Park and Lakes, 2007).

Properties	**Values**
Elastic modulus (GPa)	40-117
Compressive strength (MPa)	294
Boding strength (Mpa)	147
Hardness (Gpa)	3.43
Density (g/cm^3)	3.16

1.3 Porous Hydroxyapatite (HA)

Porous HA is important to get a strong bonding to the bone because the pore can give a mechanical interlock for a firm fixation of material. When bone tissue grows into the pore, it can increase strength of scaffold. It is important for the scaffold to form a secure bond with tissues by allowing new cell to grow, therefore the porosity is important to allow cell migration. In addition, porous scaffold can enhance to new bone tissue formation because the structure helps to allow the nutrient supply, gas diffusion and metabolic waste removal (Sepulveda *et al.*, 2000), which is important for cell survival and activity. Cell growth in pore can also help to prevent loosening and movement of scaffold. Swain *et al.*, (2010), have suggested that pore size should be more than 100 µm in order to get better regeneration of mineralized bone. If the pore sizes exceed 100 µm, bone grows through the channel of interconnected surface pore, thus maintaining the bone vascularity and viability. The most common techniques to make porous scaffolds are salt leaching, gas foaming, phase separation and freeze-drying (Sepulveda *et al.*, 2000).

1.4 Zirconia

Zirconia is a widely used ceramic material. Generally zirconia has characteristics of high hardness, high abrasion resistance, exceptionally strength and chemical inertness. These properties have made them a good candidate for high strength dental applications and bone implants. The zirconia biocompatibility has been evaluated and the material is considered to be osseointegrated when used as dental implants (Schulte *et al.*, 1988). The mechanical characteristics of alumina implants produced in the mid-seventies were considered inadequate for long-term loading and dental implant purposes. Therefore, the researchers focused their attention towards the use of zirconia as biomaterials and as a result zirconia is now being used as a ball head of total hip replacement (Piconi and Maccauro 1999) and developments are also in progress for its application in other medical devices (Mellinghoff, 2006). Zirconia occurs in three forms: monoclinic, cubic and tetragonal. Pure zirconia exists in monoclinic form at room temperature. By the addition of "stabilising" oxides, like CaO, MgO, CeO_2, Y_2O_3, the tetragonal and cubic phase can also be maintained at room temperature. Zirconia can thereby occurs in stabilized and partially stabilized form.

1.5 Polyvinyl Alcohol (PVA)

Polyvinyl alcohol was first prepared by Hermann and Haehnel in 1924 by hydrolyzing polyvinyl acetate in ethanol with potassium hydroxide (Eliassaf, 1972). Polyvinyl alcohol is produced commercially from polyvinyl acetate, usually by a continuous process. It is soluble in water, slightly soluble in ethanol, but insoluble in other organic solvents. Typically a 5% solution of polyvinyl alcohol exhibits a pH in the range of 5.0 to 6.5. Polyvinyl alcohol has a melting point of 180 to 190°C. It has a molecular weight between 26,300 and 30,000, and a degree of hydrolysis of 86.5 to 89%.

1.6 Polymeric Sponge Method

Polymeric sponge method consists of impregnation of a polymeric sponge with ceramic slurry followed by a thermal treatment which leads to the burning off the organic portion and to the sintering of the ceramic skeleton. The steps must be optimized carefully to obtain a porous product having the desired properties. The choice of polymeric sponge, preparation and solid content in the slurry, impregnation, thermal cycle including drying, burning out of the volatile components and sintering of the ceramic portion must be carefully controlled in this method. The residual stress and disruption to unsintered ceramic network should be avoided. The sponge should be volatile at low temperature and should not yield harmful by products upon heating. Its resiliency, hydrophobic behaviour and ability to be uniformly covered by slurry are other important properties. There are many polymeric sponge materials satisfying these requirements such as poly(urethane), cellulose, poly(vinyl chloride), latex (Montanaro *et al.*, 1998). The ceramic type is determined depending on the application and the desired properties of the final product. The ceramic slurry must be able to uniformly cover the polymeric walls, easily sintered into a dense ceramic network. A fine ceramic powder having a narrow size distribution is generally recommended with a mean particle size of a few microns. The quantity of the particles penetrating the polymeric sponge changes according to the structure of the sponge, therefore, the impregnation technique and the concentration of the slurry have to be optimized. The slurries may contain variable solid weight percent. In this study 20% and 30% were used. Higher the solid concentration, more and more viscous the slurry and this makes the particles difficult to penetrate the sponge that will cause loading decrease (Montanaro *et al.*, 1998).

Many additives can be used for improving strength performances. Binders can be used to provide strength to the ceramic structure after drying and prevent collapse during volatilization of the organic part. During the impregnation the slurry must be fluid enough to enter, fill and uniformly cover the sponge walls and then regain enough viscosity under static conditions to remain in the sponge. Impregnation is usually done by compression of the sponge in the slurry to remove air followed by free expansion of polymer in the slurry. The ceramic suspension should not be too viscous which will slow the sponge while trying to expand to its original shape. A typical cycle of polymeric sponge method is the preparation of the ceramic slurry, immersion of sponge in the slurry, impregnation and then drying. Thermal cycle applied to the impregnated foam which consists of two stages first of which is slowly decomposing and burning out of polymer support without collapsing. The second stage is sintering and densification of the ceramic powder. The whole processes are summarized in Figure 1.1.

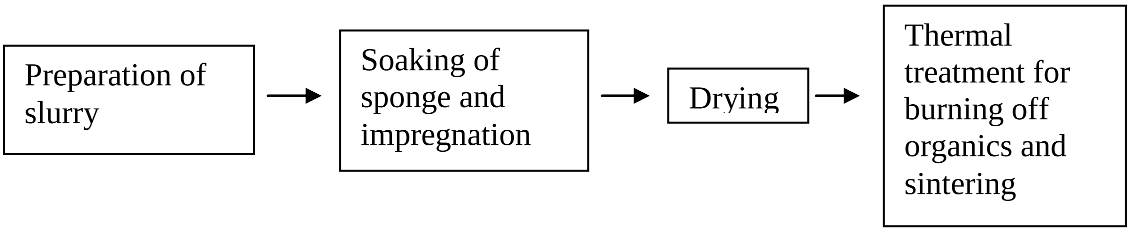

Figure 1.1: Steps involved in polymeric sponge method.

Tang *et al.*, (2008) reported that quality and properties of scaffold strongly depend on starting precursors and fabrication process. For example, in sponge structure show a reticulate macro porous structure and uniformly distribution of pore

(Jo *et al.*, 2008). Therefore, polymeric sponge method offers an advantage to make a porous scaffold. The significant feature of polymeric sponge method is uniform dispersion of ceramic powder within the polymeric structure. In addition, the polymeric sponge method receives particular attention because it can provide very high porosity with good interconnection between pore (Chen *et al*., 2006). These properties are important to promote bone ingrowths and the vascularization of newly formed tissue (Karageorgiou and Kaplan, 2005).

1.7 Bone

Bone is a type of dense connective tissues with mineralized extracellular matrix (ECM) composed of calcium phosphate and collagen (Table 1.2). Bone grafting is a surgical procedure that replaces missing bone that may have occurred due to trauma or disease. Normally, hydroxyapatite is used as a component to replace natural bone due to the good biocompatibility and eosteoconductivity (Le Gorez, 2002). However, HA has disadvantage for tissues engineering application because of brittleness and the processing to form highly porous structure is limited. To overcome the inferior mechanical properties of HA we have used zirconia to improve mechanical strength.

Table 1.2: Composition of bone (Park, 1992).

Component	**Amount (%)**
Hydroxyapatite	69
Organic matrix	22
Collagen	90-96 of organic matrix
Others	4-10 of organic matrix
Water	9

1.8 Tissues Engineering

Tissues engineering (TE) is a field which applies the principle of engineering and life sciences to build biological substances that restore, maintain or improve tissues function. In this case study, TE principles are used to develop a material which is suitable to replace or repair damaged bone tissue. Bone tissue engineering involves transplantation and biomaterial implantation (Langer and Vacanti, 1993), which can be achieved by several techniques in tissues engineering (Ma, 2004):

- Replacement of the damaged cell with isolated cell or cell substitutes that can supply a desired function
- Use of delivery of tissues
- Uses scaffold

Some of the basic requirements for tissue engineering involving scaffolds are listed in Table 1.3.

Table 1.3: Requirement for ceramic- bone tissues engineering scaffold (Swain 2010).

Property	**Factor**
Material property	Chemical composition (purity,crystallinity) Powder processing route Sintering parameter
Porous structure property	Pore size and distribution, pore shape, porosity interconnection.
Tissues interaction	Bio-compatibility, resorption rate, degradation behavior
Geometric property	Sample size, volume and weight

1.9 Problem Statement

There are biocompatible metallic materials such as stainless steel, titanium, aluminum, vanadium, cobalt, chromium and nickel that are frequently used as implanting materials to replace damaged bone or to provide support for healing bones or bone defect (Davis, 2003). However, the main disadvantages of metallic biomaterials are their lack of biological recognition on the material surface and release of toxic metallic ions and or particle through corrosion or wear leading to imflammatory cascades and allergic reaction, which results in implant failure and loss of surrounding tissue (Alvarez and Nakajima, 2009). For example the excessive cobalt may lead to polycythemi, hypothyroidism, cardiomyopathy and carcinogenesis. Aluminum also has it drawbacks where it has been associated with anemia, osteomalacia, and neurological dysfunction. Titanium often regarded as inert material has been associated with pulmonary disease (Ratner, 2004).

The limitation of those materials was overcome by the use of scaffolds made of synthetic bioceramics such as HA. The porous form of hydroxyapatite with interconnected pores and high degree of porosity can provide a favorable environment for bone ingrowth and osseointegration (Jo *et al*., 2008). Pores are important, they are conduits for blood flow (blood is generated in bone narrow) and they allow bones to be strong without being too heavy (Ain *et al*., 2008).

Porous scaffold, as the basic elements of tissue engineering, is an indispensable member of tissue engineering field. Many methods are employed to produce porous structure, however, in order to obtain 3D interconnected structure, the polymer impregnating method is widely used to prepare open-cell porous scaffold with controllable pore size and highly porous structure (Zhoa *et al*., 2009). As the main inorganic phase of natural bone, HA is known to excellent bone bonding ability, osteoconductivity and some osteoinductivity with specific porous structure (Yuan *et al.*, 1999). However, the inherent brittleness and poor mechanical properties of HA bioceramics restrict the applications in load bearing positions. Therefore, it is necessary to develop a substitute for HA bioceramics with both excellent mechanical and biological properties. In this study, zirconia is used to enhance the mechanical properties of porous scaffold.

1.10 Scope of Study

Firstly we synthesised of HA/zirconia mixture with 5%, 10% and 20% zirconia. Microwave method was selected for the synthesis using calcium nitrate tetrahydrate, zirconia and di-ammonium hydrogen phosphate as starting materials. The phase composition of the HA/zirconia mixture was determined using X-ray diffraction (XRD).

Scaffolds were prepared using sponge method with 20% and 30% powder loading. The slurries were prepared by suspending HA/zirconia powder, 3% of polyvinyl alcohol as binder and distilled water. The sponges were soaked in the prepared slurry, dried at 80°C for 24 hours in an oven followed by sintering in a muffle furnace at 1000°C for 2 hours. In order to improve the strength of the scaffold, the prepared scaffolds were soaked in a mixture of poly-L-lactic acid (PLLA)/HA (10%). Scanning electron microscope was used to study the morphology of the scaffold and compression testing was carried out to determine the compression strength of the scaffold.

1.11 Objectives

The objectives of the study are:

I. To synthesise hydroxyapatite (HA)/5%, 10%, 20% zirconia mixtures by microwave method.
II. To prepare porous HA/zirconia scaffolds by the sponge polymeric method.
III. To enhance the mechanical properties of ceramic scaffold.
IV. To characterize the ceramic scaffold.

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