SYNTHESIS AND CHARACTERISATION OF MESOPOROUS HYDROXYAPATITE BIOCERAMICS

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by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BET	Brunauer-Emmett-Teller
BJH	Barrett-Joyner-Halenda
BG	Bioactive glass
C10TAB	Decyltrimethylammonium bromide
CaP	Calcium phosphate
CHA	Carbonate apatite
CMC	Critical micelle concentration
DHA	Dense hydroxyapatite
DI	Deionised
FA	Fluoroapatite
FTIR	Fourier transform infrared
HA	Hydroxyapatite
MBG	Mesoporous bioactive glass
MCM-41	Mobil Composition of Matter No. 41
MRTD	Maximum recommended therapeutic dosages
MSN	Mesoporous silica nanoparticles
PDF	Powder diffraction file
PHA	Porous hydroxyapatite
PSD	Pore size distribution
SBA-15	Santa Barbara Amorphous No. 15
SBF	Simulated body fluid
SEM	Scanning electron microscope
TCP	Tricalcium phosphate
TEM	Transmission electron microscope
TGA	Thermogravimetry analysis
THR	Total hip replacement
TPV	Total pore volume
UV-Vis	Ultraviolet and visible
XRD	X-ray diffraction
YAS	Yttria aluminosilicate
YSZ	Yttria-stabilised zirconia

LIST OF SYMBOLS

$C_{t\text{-}corr}$	Corrected concentration at time t
C_t	Apparent concentration at time t
I_{300}	Intensity of (300) reflection
M_r	Relative molecular weight
V	Total volume of dissolution medium
v	Volume of sample taken
$V_{112/300}$	Intensity of hollow between (112) and (300) diffractions
X_c	Fraction of crystalline phase

LIST OF PUBLICATIONS

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SINTESIS DAN PENCIRIAN BIOSERAMIK HIDROKSIAPATIT BERLIANG MESO

ABSTRAK

Hidroksiapatit (HA) berliang meso dengan saiz liang yang lebih kecil dalam julat nano dan taburan saiz liang yang lebih sempit merupakan calon yang lebih sesuai untuk memperbaiki profil pelepasan ubat dengan mempamerkan pelepasan yang lebih lestari dan seragam. Kajian ini dijalankan untuk menyiasat keupayaan pengisian dan pelepasan ubat bagi HA berliang meso, yang disintesis melalui suatu mekanisma penghimpunan-diri antara HA dan surfaktan berkation desiltrimetilammonium bromida (C10TAB) dengan kaedah pemendakan. Beberapa parameter seperti teknik mengawal pH, tempoh penuaan dan nisbah air telah dicuba semasa sintesis untuk mengkaji kesan masing-masing terhadap sifat kebolehjerapan HA. Pelbagai teknik pencirian termasuk pembelauan sinar-x, penjerapan gas nitrogen dan mikroskopi imbasan elektron telah digunakan untuk mengkaji struktur dan ciri-ciri liang bagi HA berliang meso. Kombinasi parameter yang terdiri daripada nisbah air (larutan C10TAB-phosphate dan calcium) 140 : 20 ml dengan pengawalan pH berterusan dan tempoh penuaan 24 jam didapati telah memberikan ciri-ciri liang dan kebolehjerapan terbaik. HA berfasa tulen dengan kelebaran liang 2 – 6 nm dan luas permukaan 73.3 m^2/g telah berjaya disintesis menggunakan parameter-parameter tersebut. Ujian penghantaran ubat *in vitro* telah dijalankan dengan menggunakan ibuprofen sebagai ubat dan HA berliang meso sebagai pengangkut. HA berliang meso tersebut telah mempamerkan kapasiti pengisian ubat sebanyak 45.48 mg/g dan berupaya melepaskan ubat secara berterusan selama 52 jam. Selain itu, HA berliang meso dalam bentuk granul sfera berdiamater 1 – 25 μm juga telah berjaya disintesis melalui teknik sembur-kering.

SYNTHESIS AND CHARACTERISATION OF MESOPOROUS HYDROXYAPATITE BIOCERAMICS

ABSTRACT

Mesoporous hydroxyapatite (HA) with smaller pore size in the nano range and with narrower pore size distribution is a better candidate to provide a more favourable drug release profile by exhibiting a more uniform and sustained release. This research was conducted to investigate the drug impregnation and release capability of mesoporous HA synthesised via a self-assembly mechanism between HA and cationic surfactant decyltrimethylammonium bromide (C10TAB) through a co-precipitation route. Different parameters such as pH-control technique, ageing duration, and water ratio were attempted during the synthesis to investigate their respective influence on the sorption properties. Various characterisations techniques including x-ray diffraction, nitrogen gas adsorption and scanning electron microscopy were carried out to examine the structure and pore characteristics of the mesoporous HA. It was found that the combination of parameters which rendered the best pore characteristics and sorption properties consisted of water ratio (C10TAB-phosphate and calcium solution) of 140 : 20 ml with continuous pH-control and 24-hour ageing. Using these parameters, a phase-pure HA with pore width 2 – 6 nm and surface area 73.3 m^2/g was synthesised. An *in vitro* drug delivery test was also conducted using ibuprofen as a drug and mesoporous HA as a carrier. The mesoporous HA showed a drug impregnation capacity of 45.48 mg/g and was able to release drug continuously for 52 hours. In addition, mesoporous HA in the form of spherical granules with diameter 1 – 25 μm were also successfully produced through a spray-drying method.

CHAPTER 1

INTRODUCTION

The emergence of the term 'bioceramic' was brought about by a revolution that involved the innovative use of specially designed ceramics for the repair, reconstruction, and replacement of diseased or damaged parts of the body (Albee and Morrison, 1920; Boutin, 1972; Hulbert et al., 1970 and 1972). Examples of bioceramics include ceramic materials such as calcium phosphates (e.g., hydroxyapatite, tricalcium phosphate, carbonate apatite, fluoroapatite), silica, bioactive glass, zirconia, and alumina. Bioceramics may be bioinert (e.g., alumina and zirconia), resorbable (e.g., tricalcium phosphate), bioactive (e.g., hydroxyapatite, bioactive glasses, and glass-ceramics), or porous for tissue ingrowth (e.g., hydroxyapatite-coated metals) (Hench, 1998).

Hydroxyapatite (HA) is one of the calcium phosphate ceramics which has high bioactivity and biocompatibility with human bone tissues as it is part of the bone mineral composition (Shackelford, 2005). Due to this, it has been studied extensively and used as a bone scaffold and drug delivery system. HA has been used widely in the medical field and dentistry. For instances, HA can be used for dental implants, periodontal pocket obliteration, alveolar ridge augmentation, maxillofacial reconstruction, spinal surgery, and otolaryngological (Hench and Wilson, 1993). In addition, HA also can be used for delivering drug in treating osteomyelitis (Itokazu et al. 1995 and 1998) and cancer (Uchida et al., 1992).

Later, porous bioceramics were found to be a more potential scaffold (compared to dense bioceramics) as bone implants. Although the formation of pores in bioceramics may adversely affect mechanical properties, the advantages provided by the pores are crucial in repairing bone defects. The porosity within certain bioceramics can be beneficial to *in vivo* bone bonding ability due to: (a) larger surface area can induce higher bioactivity and results in a higher tendency to bioresorb, (b) interconnected pores (if available) can provide a framework for bone growth into the matrix of the implant and lead to better fixation with surrounding tissues which can increase further bone growth, (c) interconnected porosity functions as an organisation of vascular canals for transporting blood and nutrition to the bone (Nandi et al., 2009).

1.1 Dense Versus Porous Hydroxyapatite

Both dense and porous HA can be used for biological implants. Both of them are used for different purposes and also provide different advantages.

The use of dense HA (DHA) ceramic offers a greater mechanical resistance and limited degradation, therefore making it a desirable substitute for hard bone such as cortical bone. However, formation of fibrous tissue (instead of vascularised tissue) was observed in the bone/implant interface (Zhang and Vecchio, 2006). As for the porous HA (PHA), its porosity gives rise to better osteoconductive property which is essential for bone tissue growth. Nevertheless, because of their low resistance to mechanical stress, PHA ceramics were only indicated for use in non-load-bearing area (Andrade et al., 2002).

The study of Han et al. (2004) reported that PHA ceramic was only used as cancellous bone graft substitute materials in non-load-bearing situations. Although a DHA ceramic has more superior mechanical performance than a porous ceramic, its applications in load-bearing situations such as artificial joints have been restricted by its low toughness and low flexural strength. Furthermore, the bone ingrowth property of PHA is much better than that of DHA. Since pore size and porosity can adversely affect the mechanical properties of HA, a balance between the needs for osteoconductivity and favourable mechanical properties must be maintained.

1.2 Mesoporous Hydroxyapatite

Mesoporous (pore width between 2 and 50 nm) HA has been studied since the 21st century because it was believed that its combination of superior pore characteristics, high biocompatibility, and high bioactivity would lead to a breakthrough when being applied as a drug delivery system (Li et al., 2008; Yao et al., 2003). On the other hand, macroporous (pore width > 50 nm) HA was reported to exhibit 'burst' release profile of drug (Radin et al., 2005), which released unnecessarily large amount of drug at the initial stage. This could shorten the drug release duration of the HA. Therefore, mesopores, instead of macropores, were desired because they can create larger surface area for drug adsorption and provide better pore characteristics for better release profile.

Other than that, in comparison to mesoporous silica which exhibited superior pore characteristics and has been widely studied for its drug delivery behaviour

(Trewyn et al., 2004; Slowing et al., 2008), mesoporous HA might be more suitable in treating bone diseases because its higher bioactivity and osteoconductivity would help to heal the tissues at the defective part while delivering drug, whereas the bioinertness of silica would not allow it to contribute to the recovery of bone tissues. In short, mesoporous HA could serve not only as a drug delivery system, but also as a scaffold simultaneously.

The methods to synthesise mesoporous HA include soft-templating (Li et al., 2008; Yao et al., 2003). The soft-templating method proposed by Yao et al. (2003) involves the use of surfactant to form micelles template for creating mesopores in the HA. Li et al. (2008) showed that higher ageing duration (120 – 160 °C) could create smaller pore width (*ca.* 2 nm) using the proposed procedure. To date, the synthesis of mesoporous HA bioceramics with pore characteristics similar to that of mesoporous silica is a great challenge. However, such studies are worth attempting to investigate the potential of mesoporous HA in various applications.

1.3 Problem Statements

HA could be synthesised by different pH-control techniques i.e., initial control (Li et al., 2008; Yao et al., 2003) and continuous control (Li et al., 2009; He et al., 2009; Mobasherpour et al., 2007; Pang and Bao, 2003). However, there was no study carried out to compare the effect of both techniques on the pore characteristics of mesoporous HA. Hence, both techniques were adopted in this study to compare their effect.

Different ageing durations were reported to create different pore size distribution in zirconia, titania, and silica (Suh et al., 2000; Suh and Park, 2002). However, the effect of ageing duration on the mesopores of HA has not been reported yet. Therefore, the effect of ageing duration on the surface area and pore size distribution of mesoporous HA should be investigated to provide a better understanding.

Previous studies conducted to synthesise mesoporous HA through co-precipitation method had used water in the amount of 100 ml to prepare decyltrimethylammonium bromide (C10TAB)-phosphate solution and 60 ml to prepare calcium solution (Li et al., 2008; Yao et al., 2003). However, no particular reason in choosing this ratio was explained. Thus, various water ratios were used during synthesis to study their effect on the pore characteristics of HA.

Spherical HA granules were preferred over the one with irregular morphology since the latter was reported to cause inflammatory reactions (Laquerriere et al., 2003; Paul and Sharma, 1999). However, the synthesis of spherical mesoporous HA granules through a spray-drying technique has not been attempted before. Thus, such synthesis was carried out to study the characteristics of spherical mesoporous HA granules.

1.4 Objectives

1. To synthesise mesoporous HA using soft-templating method and to obtain spherical mesoporous HA granules using a spray-drying technique.

2. To study the effect of various synthesis parameters (e.g., pH-control technique, ageing duration, and water ratio) on the sorption properties of mesoporous HA.
3. To study the *in vitro* drug (ibuprofen) delivery behaviour of mesoporous HA.

1.5 Outline of Study

This study basically consisted of four main parts.

I. Synthesis of mesoporous HA with various parameters.
II. Characterisation of mesoporous HA
III. *In vitro* delivery of ibuprofen drug using mesoporous HA
IV. Synthesis of spherical mesoporous HA granules.

In Part I, the synthesis method was adopted from that of Yao et al. (2003) and Li et al. (2008), which involved the self-assembling between HA and cationic surfactants. The parameters studied included water ratio, pH-control technique and ageing duration. In Part II, the synthesised mesoporous HA was characterised to study its phase, sorption properties, surface morphologies, and functional groups. In Part III, *in vitro* ibuprofen drug delivery tests were conducted using synthesised mesoporous and synthesised dense HA. The results for both samples were compared to study the effect of pores in drug delivery. The reasons to choose ibuprofen drug were, firstly, the molecular size of ibuprofen is 1.0 x 0.6 nm (Vallet-Regi et al., 2001), which could be fit into mesopores. Secondly, ibuprofen is a common and highly available pharmaceutical drug which is being used as an analgesic and also as an anti-inflammatory drug (e.g., for medical treatment of rheumatoid arthritis)

(Manzano et al., 2008). Lastly, the high availability of studies on ibuprofen delivery by carriers based on mesoporous silica (Du and He, 2010; Jin and Liang, 2010; Izquierdo-Barba et al., 2009; Manzano et al., 2008; Qu et al., 2006; Vallet-Regi et al., 2001) could give a broader view to provide better understanding in this field. As for Part IV, a spray-drying method was adopted to produce spherical mesoporous HA granules and their characteristics were compared with those of non-spray-dry.

CHAPTER 2

LITERATURE REVIEW

2.1 Biomaterials

A biomaterial is defined as any material used to make devices to replace a part or a function of the body in a safe, reliable, economic, and physiologically acceptable manner (Park and Lakes, 2007). A biomaterial is a synthetic material used to function in intimate contact with living tissue or to replace part of a living system. A biomaterial is different from a biological material, such as bone, that is produced by a biological system (Shi and Jiang, 2006). Biomaterials is an interdisciplinary research topic which involves the knowledge of three different fields: (1) materials science and engineering processing-structure-property interrelationship of synthetic and biological materials including metals, ceramics, polymers, composites, tissues; (2) biology and physiology cell and molecular biology, anatomy, animal and human physiology, and (3) clinical sciences dentistry, ophthalmology, orthopaedics, plastic and reconstructive surgery, cardiovascular surgery, neurosurgery, immunology, histopathology, experimental surgery, veterinary medicine and surgery (Shi, 2006). Since the goal of using biomaterials is to improve human health by restoring the function of natural living tissues and organs in the body, the knowledge on the relationships between the properties, functions, and structures of biomaterials must be mastered. Therefore, the subject of biomaterials basically includes three aspects of study i.e., biological materials, implant materials, and interaction between the two in the body (Park and Lakes, 2007).

2.1.1 Requirements for Biomaterials

In order to use a biomaterial effectively and successfully, attentions have to be given to the appropriate material selection, engineering design and manufacturing process. It is extremely important to select the suitable material to provide the appropriate properties as well as being biocompatible, even though proper design and manufacture are also essential. Both the influence of mechanical and chemical factors can also be critical, e.g., causing fatigue, corrosion fatigue, stress corrosion, wear, and fracture (William, 1991).

Aside from the requirements mentioned above, other factors that must be considered are: the health condition of the recipient, and the competency of the surgeon who implants and monitors its progress. Several criteria for choosing an implant include:

1. Acceptance of the implant to the tissue surface, i.e., biocompatibility
2. Pharmacological acceptability (non-toxic, non-allergenic, non-immunogenic, non-carcinogenic)
3. Chemically inert and stable (no time-dependent degradation)
4. Adequate mechanical strength
5. Adequate fatigue life
6. Sound engineering design
7. Proper weight and density
8. Relatively inexpensive, reproducible, and easy to fabricate and process for large-scale production (Park and Lakes, 2007)

2.1.2 Examples of Biomaterials

Biomaterials are divided into several categories based on their structural, chemical, and biological characteristics, for example, as in ceramics, glasses, and polymers with a varied degree of bioactivity (Shi, 2006). While biomaterial applications involve metals, ceramics, polymers and composites, they are divided basically into three types. These are

(i) inert or relatively inert with minimal host response

(ii) bioactive which actually stimulates bonding to the surrounding tissue and

(iii) biodegradable which resorb in the body over a period of time (William, 1991).

Table 2.1 listed some of the advantages, disadvantages, and applications of four groups of synthetic (manmade) materials used for implantation (Park and Lakes, 2007).

2.2 Bioceramics

"Ceramic" is a term translated from the Greek *keramos,* which means pottery or burned stuff. Ceramics are composed of inorganic and non-metallic materials and include pottery, porcelain, refractory materials, clay products, abrasives, porcelain enamels, cements, glasses, non-metallic magnetic materials, ferroelectrics, and manufactured single crystals (Kingery et al., 1976).

About six decades ago, a revolution occurred in the use of ceramics to improve the quality of life (Hench, 1998). The revolution involved the innovative use of specially designed ceramics for the repair, reconstruction, and replacement of

Table 2.1 Class of materials used in human body (Park and Lakes, 2007)

Materials	Advantages	Disadvantages	Example applications
Polymers (nylon, silicone, rubber, polyester, polytetrafluoroethylene)	Resilient Easy to fabricate	Not strong Deforms with time May degrade	Sutures, blood vessels, soft tissues, hip socket, ear, nose
Metals (Ti and its alloys, Co-Cr alloys, Au, Ag stainless steels)	Strong, tough, ductile	May corrode Dense Difficult to fabricate	Joint replacements, dental root implants, pacer and suture wires, bone plates and screws
Ceramics (Alumina, zirconia, calcium phosphates including HA)	Very biocompatible	Brittle Not resilient Weak in tension	Dental and orthopaedic implants
Composites (carbon-carbon, wire- or fibre-reinforced bone cement)	Strong, tailor-made	Difficult to make	Bone cement, dental resin

diseased or damaged parts of the body (Albee and Morrison, 1920; Boutin, 1972; Hulbert et al., 1970, 1972). Hence, the term 'bioceramics' emerged. 'Bioceramics' are defined as any ceramic, glass or glass-ceramics used as a biomaterial, which is a material intended to interface with biological systems to evaluate, treat, augment, or replace any tissue, organ, or function of the body (Williams, 1999). Similarly, the term 'bioceramics' also refers to biocompatible ceramic materials that are applicable for biomedical or clinical uses (Tanaka and Yamashita, 2008). Bioceramics can be polycrystalline (alumina or hydroxyapatite), bioactive glass, bioactive glass-ceramic, or bioactive composite (polyethylene–hydroxyapatite) (Hench, 1998).

2.3 Types of Bioceramics

There have been a number of bioceramics which are well-known for their potential in biomedical applications. Among them are silica, alumina, zirconia, glass-ceramics, and calcium phosphates.

2.3.1 Silica

Silica is a bioceramic which plays important roles in bioactive glass fabrication (Jones, 2008), allowing apatite nucleation (Cho et al., 1996), coating on titanium and hydroxyapatite (Borum and Wilson, 2003; Yoshida et al., 1999), and drug delivery (Kortesuo et al., 2000). However, there was no report on the formation of bond between pure silica and surrounding tissues so far. Thus, pure silica is categorised as a bioinert material which does not play any major role in the repair of bone defect. This is supported by the finding of Klein et al. (1995) which confirmed that silica content exceeding 60 mol% will give rise to bioinert property. Nevertheless, silica is bioresorbable (Ahola et al., 2001) and mesoporous silica nanoparticles exhibit a high biocompatibility at concentrations adequate for potential pharmacological applications, unlike amorphous silica which showed cytotoxicity towards mammalian red blood cells (Slowing et al., 2009).

2.3.2 Alumina

Alumina is widely used for biomedical applications due to its good mechanical properties from excellent strength, promising fracture toughness, high wear resistance, good biocompatibility, to excellent corrosion resistance (De Aza et

al., 2002). High-density and high-purity (>99.5%) alumina (Al_2O_3) was the first bioceramic widely used in the medical field (Hench, 1998). However, it has a much lower tensile strength (259 MPa) than compressive strength (2965 MPa) due to its brittleness (i.e., it cannot undergo plastic deformation like metals and plastics) (Park, 2008), which was a disadvantage for orthopaedic load-bearing applications. Boutin (1972) was the first to use alumina ceramic materials as bearing surfaces in artificial hips in the early 1970s. Most of the alumina used for implant applications was either a polycrystalline solid of high density and purity or an artificially grown colourless single crystal similar to sapphire or ruby (Park, 2008).

2.3.3 Zirconia

Phase-stabilised zirconia has become a popular alternative to alumina as a structural ceramic (Walpole et al., 2009), especially in total hip replacement (THR) applications, because of its substantially higher fracture toughness (10.5 $MPa.m^{1/2}$) (Park, 2008). The fracture strength of zirconia ceramic heads was approximately double that of alumina heads (5.4 $MPa.m^{1/2}$) (Drouin et al., 1997). In order to achieve better mechanical properties, yttrium is added into the crystal structure of zirconia in the form of yttrium (III) oxide to form yttria-stabilised zirconia (YSZ). In orthopaedic implants, YSZ is commonly used due to its combination of outstanding mechanical properties, biocompatibility, and wear behaviour against polyethylene. Zirconia has been receiving attention due to its high tensile strength and fracture toughness. The superior properties of zirconia allowed it to manufacture femoral heads for total hip prostheses that were smaller than the present generation of alumina heads (Hench, 1998). The use of zirconia (ZrO_2) for biomedical applications

was first reported by Helmer and Driskell (Helmer and Driskell, 1969) in 1969. The first paper reporting the use of zirconia to manufacture ball heads for THR, which is the current main application of this bioceramic, was published by Christel et al. (1988) in 1988. Overall, by 2006, more than 600,000 zirconia femoral heads had been used as an implant worldwide, mainly in the US and in Europe (Chevalier, 2006).

2.3.4 Glass-ceramics

Glass-ceramics were developed by Stookey in the early 1960s. Glass-ceramics are actually polycrystalline ceramics made by controlled crystallisation of glasses. Initially, they were applied in photosensitive glasses in which copper, silver and gold were precipitated by ultraviolet light irradiation. Such precipitation facilitated the nucleation and crystallisation of the glass into a fine grained ceramic which possess superior mechanical and thermal properties (Park and Lakes, 2007). Two types glass-ceramic have been developed as bioceramics i.e., Bioglass® and Ceravital® (Blencke et al., 1978; Hench and Pachall, 1973; Ogino et al., 1980; Piotrowski et al., 1975). The compositions of the two glass-ceramics are rather similar in terms of SiO_2 content (Table 2.2) but Ceravital® is composed of Al_2O_3, TiO_2, and Ta_2O_5 which are important to control the dissolution rate (Park and Lakes, 2007).

Compared to hydroxyapatite and tricalcium phosphate, bioactive glasses have an advantage of being able to be used as a substrate stronger than cortical bone and they also possess the ability to form strong chemical bonds with bone. For instance, a

Table 2.2 Compositions of Bioglass® and Ceravital® Glass-Ceramics (Park and Lakes, 2007)

Type	Code	SiO_2	CaO	Na_2O	P_2O_5	MgO	K_2O
Bioglass							
	42S5.6	42.1	29.0	26.3	2.6	-	-
	(45S5)46S5.2	46.1	26.9	24.4	2.6	-	-
	49S4.9	49.1	25.3	23.0	2.6	-	-
	52S4.6	52.1	23.8	21.5	2.6	-	-
	55S4.3	55.1	22.2	20.1	2.6	-	-
	60S3.8	60.1	19.6	17.7	2.6	-	-
Cervital*							
	Bioactive	40-50	30-35	5-10	10-15	2.5-5.0	0.5-3.0
	**Non-bioactive	30-35	25-30	3.5-7.5	7.5-12.0	1.0-2.5	0.5-2.0

*The Ceravital composition in weight % while the Bioglass compositions are in mol %.
**In addition Al_2O_3 (5.0-15.0), TiO_2 (1.0-5.0) and Ta_2O_5 (5.0-15.0) are added.

glass-ceramic containing apatite and wollastonite exhibited better mechanical properties than bone tissue and was able to bond chemically with living bone tissue (Ono et al., 1990; Sautier et al., 1994). Similar to other glasses and ceramics, the major disadvantage of the glass-ceramic is its brittleness. Besides that, the mechanical strength of glass-ceramics cannot be substantially improved because varying its composition might compromise its biocompatibility. Thus, they are not suitable to be used as load-bearing implants such as joint implants. However, they can be used as dental restorative composites, coating material, and fillers for bone cement (Park and Lakes, 2007).

2.3.5 Calcium phosphates

Calcium phosphate (CaP) can be crystallised into mono-, di-, tri-, and tetra-calcium phosphate, hydroxyapatite (HA), carbonate apatite, fluorapatite, α-tricalcium phosphate (α-TCP), and β-tricalcium phosphate (β-TCP). Such crystallisation depends on the Ca/P ratio, the presence of water and impurities, and the synthesis temperature.

HA is an important biomaterial (with a Ca/P ratio of 1.67) present in bones and teeth. In fact, it comprises the primary mineral content of bone (43 wt%) (Shackelford, 2005), which implies that HA is highly biocompatible in nature. Dense HA has been used in orthopaedics or for bone substitutes (Asazuma et al., 2005; Mangano et al., 2008; Park, 2008) and dental implants (Layman and Ardoin, 1998). Although HA is not osteoinductive, it possesses good osteoconductive properties as well as a remarkable ability to bind directly to bone (Chen et al., 2008). Among the calcium phosphate bioceramics, stoichiometric HA dissolves and precipitates at the lowest rate (Shi and Wen, 2006). Macroporous HA has been studied intensively since the 1980s. However, the study of mesoporous (pore width between 2 and 5 nm) and microporous (pore width below 2 nm) HA for *in vitro* or *in vivo* testing is rather new, although they have a great potential to serve as drug delivery systems. There are also some other types of apatite ceramics, such as carbonate apatite (CHA) and fluoroapatite (FA). However, among the three porous apatites, interest is mainly focused on porous HA at present.

Tricalcium phosphate (TCP) has a Ca/P ratio of 1.50, which is similar to that of amorphous biological precursors to bone (Bodde et al., 2007). Despite their

similarity in composition, TCP and apatites are different, as the former is not found in natural bone. TCP has been developed as a bioactive and bioresorbable bone substitute due to its higher solubility compared to HA (Shi and Wen, 2006). In general, TCP is classified into α-tricalcium phosphate (α-TCP) and β-tricalcium phosphate (β-TCP). β-TCP can be converted to α-TCP when it is heated to 1125 °C (Rey et al., 2008). More attention has been focussed on β-TCP than on α-TCP for biomedical applications due to the instability (Rey et al., 2008) and cytotoxicity (Dos Santos et al., 2002) of α-TCP. However, α-TCP can be converted into apatite through a hydrothermal treatment (Wakae et al., 2008). Macroporous β-TCP has been studied intensively as a scaffold for bone growth and as a matrix for various proteins and growth factors. Nevertheless, application of pure β-TCP to bone defect repair are limited due to the inferior mechanical properties (compressive strength, bending strength, Young's modulus) of β-TCP when compared to HA (Hench, 1998).

HA, CHA, and FA are different due to the presence of different anion, i.e., hydroxyl (OH^-), carbonate (CO_3^{2-}), and fluoride (F^-), respectively. There are basically two types of CHA i.e., type A and type B, depending on the position of the carbonate in the apatite lattice. For type A, the carbonate ions occupy the hydroxyl site. For type B, the ions occupy the phosphate (PO_4^{3-}) site. In comparison to HA, CHA has a composition closer to that of bone mineral (LeGeros, 1981) and has higher resorbability (LeGeros and Tung, 1983). This implies that CHA can serve as a more favourable bone scaffold which can be replaced completely by new bone. On the other hand, FA is formed when the F^- occupy the hydroxyl site of apatite. FA has higher thermal and chemical stability, and lower resorbability compared to HA (Kim et al., 2004A; Krajewski et al., 1990). Besides that, FA also exhibits caries-inhibiting

property which does not affect its biocompatibility (LeGeros et al., 1983). However, FA is too stable and lacks biological properties and, therefore, might not be a good biomaterial when used alone (Downes et al., 1995). Despite that, FA can be used as an intermediate layer inserted between HA and zirconia. This will be able to prevent any direct contact between both of them which will lead to the decomposition of HA to TCP (Kim et al., 2003).

2.3.6 Hydroxyapatite

Hydroxyapatite (HA) is a member of the apatite family. The term "apatite" originated from the Greek *apatê*, which means deceit or deception. It bears such a meaning due to its diversity of form and colour (McConell, 1973). HA has a specific crystallographic structure: hexagonal $P6_3/m$ space group (Albee and Morrison, 1920; Andrew et al., 1962; Aoki et al., 1972). However, pure HA is different from biological apatites whereby biological apatites contain important minor substituents (e.g., CO_3^{2-}, Na^+, Mg^{2+}) and are more accurately described as carbonate apatite (CHA), approximated by the formula $(Ca,Mg,Na)_{10}(PO_4,HPO_4,CO_3)(OH)_2$ (Ducheyne et al., 1980; Holmes, 1979). Besides constituting the inorganic or mineral phases of normal calcified tissue (teeth and bones), biological apatites are also found in some pathologic calcifications (dental calculus, heart calcifications, urinary stones, soft-tissue calcifications) (Ducheyne et al., 1980; Evans, 1973; Holmes, 1979).

2.3.6.1 Chemical Properties of HA

The rate of HA dissolution *in vivo* depends on factors including degree of crystallinity, crystallite size, processing conditions (temperature, pressure, and partial water pressure), and porosity (LeGeros, 1993; LeGeros and Tung, 1983; Moreno et al., 1997). The solubility of sintered HA is lower than the unsintered one. HA is insoluble in an alkaline solution while soluble in an acidic one and slightly soluble in distilled water. These solubility properties are closely related to the biocompatibility of HA with tissues and its chemical reactions with other compounds. HA reacts actively with proteins, lipids, as well as other inorganic and organic species (Park, 2008). The rate of solubility is 0.1 mg/year in subcutaneous tissue (Katz and Harper, 1986).

The solubility of apatite is also affected by substitutions in the apatite structure. For example, compared with unsubstituted apatites prepared by precipitation or hydrolysis method, strontium, magnesium or carbonate substitution causes an increase in solubility (LeGeros, 1981; LeGeros and Tung, 1983) while fluoride substitution causes a decrease in solubility (LeGeros, 1981; Moreno et al., 1997).

2.3.6.2 Mechanical Properties of HA

The mechanical properties of a dense HA are influenced by the properties of the apatite powder, the compression and sintering conditions, and porosity. Several mechanical properties (e.g., compressive strength) degrade with increasing amount of porosity (Denissen et al., 1985). The mechanical properties are highly dependent

on the preparation of the apatite powder (Li et al., 2002). The difference in preparation methods causes difference in composition and in grain size (small grain size tends to give greater fracture toughness). Flexural strength and fracture toughness of dense HA was higher in wet than in dry conditions (Denissen et al., 1985). Meanwhile, higher sintering temperature will increase the density, grain size, compressive, flexural, torsional strength and moduli of elasticity in compression (Aoki, 1994; De Groot, 1983; Jarcho, 1981). The fracture toughness of HA ceramic increased for HA sintered from 1100 to 1150 °C, but made no significant difference from 1150 to 1250 °C, and decreased at sintering temperature above 1250 °C (Aoki, 1994). The modulus of elasticity of HA is 40 – 117 GPa compared with that of cortical bone of 12 – 18 GPa (Aoki, 1994).

2.3.6.3 Biological Properties of HA

The biological properties will be discussed based on several aspects. They include *in vitro* cell response and *in vivo* tissue response (bioactivity, osteoconductivity, and osteoinductivity).

The cell response (proliferation, differentiation, phenotypic expression of bone markers) to various materials can be demonstrated by *in vitro* cell culture studies. Responses of osteoblast (bone-forming), osteoclast (bone-resorbing), odontoblast (dentin-forming) and periodontal (associated periodontal ligament attachment) cells to HA have been reported (Craig and LeGeros, 1999; Frondoza, 1998; Fujimori et al., 1998; Inoue et al., 2004).

In vivo tissue response to biomaterials depends mainly on: bioactivity, osteoconductivity and osteoinductivity (LeGeros, 2002). Tissue response in terms of bone ingrowth also depends on the porosity (pore size, pore structure, degree of pore interconnectivity, pore volume). Bioactivity is the ability of the material to directly 'bond' to bone through chemical interaction and not physical or mechanical attachment (Hench, 1994; Osborn and Newesely, 1980). *In vitro* and *in vivo* bioactivity have been investigated by studying the ability of the material to form CHA (similar to bone apatite) on its surface (Boyde et al., 1999; Heughebaert et al., 1988; Kokubo, 1996; LeGeros and Daculsi, 1990; LeGeros et al., 1991A). Apatite nano-crystals similar to bone apatite were formed on the surfaces of coralline HA crystals and this was associated with ceramic HA for the same amount of time of suspension in bovine serum (LeGeros et al., 1991B).

Meanwhile, osteoconductive property was exhibited by HA whereby HA was able to serve as a scaffold or template for the formation of new bone tissues along its surfaces. Osteoconductive materials allow bone cell attachment, proliferation, migration, and phenotypic expression, which bring about formation of new bone on the biomaterial and result in a strong interface (LeGeros and LeGeros, 2008). Although CaP materials are bioactive and osteoconductive, they are usually not osteoinductive (LeGeros, 2002). Despite that, some porous HA exhibited osteoinductive property (Kuboki et al., 1998; Ripamonti et al., 1992) and it was believed to be influenced by its specific geometry and optimal pore size.

2.4 Applications of Bioceramics

Bioceramics have been widely used in medical applications. Shackelford categorised the applications into three main fields, i.e., orthopaedics, dentistry, and cancer treatment (Shackelford, 2005). In orthopaedics, bioceramics were used for joint replacement (especially total hip replacement) as well as defect and fracture repair. The common bioceramics involved were alumina (Al_2O_3), partially stabilised zirconia (ZrO_2), and calcium phosphate (e.g., HA, TCP). The potential of macroporous HA in repairing bone defects was reviewed by Lew et al. (2011). In dentistry, traditional dental porcelains composed of mainly leucite and aluminosilicate have been used to repair diseased and decayed teeth. Aside from traditional porcelains, alumina ceramics, and glass-ceramic prosthetics, Bioglass® (Hench, 1998) implants also played important roles in this application. In cancer treatment, glass beads and ferromagnetic glass-ceramics have been used for internal delivery of therapeutic radiation and local thermal treatment of bone tumours, respectively. A typical example of a material used in glass beads was yttria (yttrium(III) oxide) aluminosilicate (YAS) glass. Upon neutron bombardment, yttrium formed a radioactive isotope (Y-90). Such incorporation of radioactive isotope of yttrium during glass melting caused the glass to be highly radioactive with the formation of yttria in the glass composition, which was crucial for cancer radiotherapy. Ferromagnetic glass-ceramics could be obtained by incorporating magnetite in a $CaO{\cdot}SiO_2$-based matrix. After implantation of the glass-ceramic, an alternating magnetic field was applied to create a heating effect that was able to kill cancer cells (Shackelford, 2005).

2.4.1 Macroporous Hydroxyapatite as Drug Delivery Systems

Macroporous HA was suitable to be used as an implantable drug delivery system because of its high biocompatibility, simplicity and reproducibility. Another advantage of HA drug delivery system was the possibility of creating various controlled pore sizes to control the release rate of drugs (Uchida et al., 1992). In addition, any antibiotics can be placed in the macroporous HA because there was no thermal damage to the drug (Shinto et al., 1992). Ma et al. (2008) confirmed that the property of ibuprofen was not changed in the loading and releasing processes from macroporous HA. Macroporous HA was also favoured for its sustained drug release and this was safer compared to a single administration of high concentration of drug because chances for the latter to reach the toxic level and cause side effects were high (Uchida et al., 1992). Macroporous HA was able to treat diseases like cancer (Uchida et al., 1992) and osteomyelitis (Itokazu et al., 1995 and 1998) without any complication or inhibition to tissues activities other than the tumours.

However, HA (and other calcium phosphates) exhibited 'burst' release profile, which was a disadvantage for controlled-release applications (Radin et al., 2005). Despite that, one of the effects of porosity on drug release has been confirmed in the study of Palazzo et al. (Palazzo et al., 2005) whereby lower porosity of HA showed more evident initial burst release. This behaviour was due to the tendency of the drug molecules to concentrate themselves on the external macropore walls rather than internal pores. The tendency will become higher with the decreasing of the ceramic porosity and surface area, because of the difficulty for the drug to reach the internal pores during the drug introduction to the carrier. Similar explanation was given by Kim et al. (2004B) whereby the initial burst effect was due to the abrupt release of

drugs insufficiently entrapped or loosely bound to the surface. In other words, HA with higher porosity and smaller pore size would increase the surface area and allow larger amount of drug to enter the pores. This was where the idea of using mesoporous HA started.

Although the pore characteristics of mesoporous HA were not as good as those of mesoporous silica, mesoporous HA may be a more suitable candidate in delivering drug to treat bone defects because its high bioactivity and good osteoconductivity were beneficial for the growth of bone tissues in defective part. In other words, mesoporous HA could serve two purposes at the same time i.e., to deliver drug and to be a scaffold. On the other hand, the bioinertness of mesoporous silica prohibited it from being a scaffold in treating defective bones. However, up-to-date, there is no reported study on the performance of mesoporous HA in drug delivery yet.

2.5 Mesoporous Bioceramics

After the initial study on macroporous (pore width >50 nm) bioceramics, interest was aroused gradually in mesoporous (pore width 2 – 50 nm) bioceramics to improve pore characteristics as a result from the discovery of Mobil Composition of Matter No. 41 (MCM-41) (Kresge et al., 1992). Ever since MCM-41 was discovered and had been a great success, nanoporous (pore width <100 nm) materials have received great attention as the presence of nanopores were believed to be capable of improving certain properties (e.g., drug release profile) or even rendering new properties (e.g., as a catalyst) to a material. However, it is not a simple task to create