DEVELOPMENT OF CARBONATED HYDROXYAPATITE/ POLY(L-LACTIDE)/ POLY(VINYL ALCOHOL) BIOCOMPOSITES

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DEVELOPMENT OF CARBONATED HYDROXYAPATITE/ POLY(L-LACTIDE)/ POLY(VINYL ALCOHOL) BIOCOMPOSITES

by

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I declare that the contents presented in this thesis are my own work which was done at Universiti Sains Malaysia unless stated otherwise. The thesis has not been previously submitted for any other degree.

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LIST OF ABBREVIATIONS

BGS	Bone graft substitute
CHA	Carbonated hydroxyapatite
CHN	Carbon, hydrogen, nitrogen
DP	Direct pouring
DSC	Differential scanning calorimetry
DTS	Diametral tensile strength
DW	Dropwise
EFTEM	Energy filtered transmission electron microscope
FESEM	Field emission scanning electron microscope
FTIR	Fourier transform infrared
FWHM	Full width half maximum
HA	Hydroxyapatite
ICDD	International Centre for Diffraction Data
PLA	Poly(lactide)
PLLA	Poly(L-lactide)
PVA	Poly(vinyl alcohol)
rpm	Revolution per minute
SBF	Simulated body fluid
TE	Tissue engineering
TS	Tensile strength
TGA	Thermogravimetric analysis
XRD	X-ray diffraction
XRF	X-ray fluorescence

LIST OF SYMBOLS

2θ	X-ray incident angle
Ø	Diameter
mol%	Mole percent
M_n	Number of average molecular weight
M_w	Weight average molecular weight
T_{cc}	Cold crystallization temperature
T_g	Glass transition temperature
T_m	Melting temperature
T_{max}	Temperature of maximum weight loss
T_{ons}	Onset degradation temperature
vol%	Volume percent
wt%	Weight percent

PENGHASILAN BIOKOMPOSIT HIDROKSIAPATIT BERKARBONAT/ POLI(L-LAKTIDA)/ POLI(VINIL ALKOHOL)

ABSTRAK

Hidroksiapatit berkarbonat (CHA) jenis B yang bersaiz nano telah disintesis melalui kaedah pengemulsian nano dengan menggunakan teknik titisan (DW) dan tuangan langsung (DP). Serbuk CHA daripada teknik DP mempunyai kandungan CO_3^{2-} yang lebih tinggi dan saiz yang lebih kecil berbentuk seperti sfera, berbanding dengan serbuk DW dengan bentuk yang memanjang. Selain itu, saiz partikel CHA didapati menurun dengan peningkatan kandungan CO_3^{2-}, dengan penggantian CO_3^{2-} yang maksima, iaitu 14 wt%. Selepas itu, proses penyepuhlindapan dijalankan pada suhu 300–900 °C dan diikuti oleh proses pengkarbonan. Pengekalan CO_3^{2-} jenis B yang secukupnya dan kehabluran yang lebih baik dalam sampel telah dihasilkan pada suhu optima 700°C. Bagi penghasilan biokomposit CHA dengan poli(L-laktida) (PLLA) dan/atau poli(vinil alkohol) (PVA), pembentukan ikatan hidrogen antara kumpulan hidroksil daripada CHA dan karbonil daripada PLLA telah diperhatikan, manakala tiada interaksi berlaku antara CHA dengan PVA. Apabila biokomposit CHA/PLLA/PVA telah dihasilkan, PLLA memainkan peranan sebagai agen gandingan untuk mengikat CHA dan PVA melalui ikatan hidrogen. Dari aspek mekanikal, kekuatan tegangan garis pusat (DTS) daripada biokomposit meningkat dengan peningkatan kandungan polimer dan penambahan PLLA berbanding dengan PVA. Walau bagaimanapun, CHA/PLLA/PVA menunjukkan nilai DTS yang setanding pada muatan polimer yang lebih rendah. Dari aspek bioaktiviti, biokomposit CHA/PLLA/PVA menunjukkan kadar peresapan dan pembentukan apatit yang lebih baik berbanding dengan CHA/PLLA, manakala CHA/PVA adalah rendah dengan kehilangan berat tertinggi dalam simulasi bendalir badan.

DEVELOPMENT OF CARBONATED HYDROXYAPATITE/ POLY(L-LACTIDE)/ POLY(VINYL ALCOHOL) BIOCOMPOSITES

ABSTRACT

Nanosized B-type carbonated hydroxyapatite (CHA) was successfully synthesized through nanoemulsion method, by both dropwise (DW) and direct pouring (DP) techniques. The CHA powders obtained by DP method contained higher CO_3^{2-} content with smaller near-spherical size, as compared to the one by DW with elongated shape. Moreover, the CHA particle size was found to decrease with increasing CO_3^{2-} content, with maximum CO_3^{2-} substitution of 14 wt%. Annealing followed by carbonation at cooling stage on CHA was carried out in the range of 300–900 °C. The optimum temperature of 700°C was determined from the adequate B-type CO_3^{2-} content retained and improved crystallinity of the annealed powder. In the biocomposite fabrication of CHA with poly(L-lactide) (PLLA) and/or poly(vinyl alcohol) (PVA), hydrogen bonding was deduced to form between hydroxyl group of CHA and carbonyl of PLLA, while no interaction was observed between CHA with PVA. When CHA/PLLA/PVA biocomposites were fabricated, PLLA served as coupling agent which bridged CHA and PVA via hydrogen bonding. From the mechanical aspect, diametral tensile strength (DTS) of the biocomposites was found to increase with increasing polymer loading and when PLLA was added instead of PVA. Nevertheless, CHA/PLLA/PVA biocomposites exhibited comparable DTS value at lower polymer content. In terms of bioactivity, the CHA/PLLA/PVA biocomposite showed better resorption rate and apatite formation as compared to CHA/PLLA, while CHA/PVA was low with highest weight loss in simulated body fluid.

CHAPTER 1
INTRODUCTION

1.1 Background

An adult human body has 206 bones, acting as support for the body and its movements as well as protecting vital organs such as heart, lung and brain (Wang, 2004). At nanostructural level, the smallest structure in bone includes non-collagenous proteins, fibrillar collagens and embedded hydroxyapatite crystals (Chen and Webster, 2011). However, bones are prone to injuries, diseases, defects and aging, resulting from many different causes. Pielichowska and Blazewicz (2010) stated that even though the small defects in bone can be fixed by growth of natural bone, while fractures can be self-repaired by the human body, the body is however unable to repair large defects. Consequently, bones are one of the most commonly replaced tissues in human body.

Synthetic bone graft is more preferable in bone reconstructing as compared to the biological graft as it overcomes the disadvantages of natural bone implant which gives trauma, donor site morbidity, risk of disease transmission and immune rejection (Bohner, 2010; Baino, 2011). Brydone et al. (2010) stated in their review that in the USA alone, there are an estimation of 280,000 hip fractures, 700,000 vertebral, and 250,000 wrist fractures each year at a cost of $10 billion. The global biomaterial market is estimated to increase from $44.0 billion in 2012 $88.4 billion by 2017 as reported in "Biomaterials Market [By Products (Polymers, Metals, Ceramics, Natural Biomaterials) & Applications (Cardiovascular, Orthopedic, Dental, Plastic Surgery, Wound Healing, Tissue Engineering, Ophthalmology, Neurology Disorders)] – Global Forecasts to 2017". The Malaysian Osteoporosis

Society (MOS) also expected the number of hip fracture alone to be 500,000 annually by the year 2040.

Much research work has been devoted to the production of bioresorbable surgical devices that could avoid surgical operation for their removal, thereby reducing the pain of the patients and the total cost of the treatment when compared, for example, to the use of metallic devices (Whang et al., 1995; Rokkanen et al., 2000; Mathieu et al., 2006). Stress-shielding phenomena associated with the use of rigid metallic implants could also be minimized (Bonfield, 1988b). The continuous degradation of the implant causes a gradual load transfer to the healing tissue, preventing stress-shielding atrophy and stimulates the healing and remodelling of the bone (Ignatius et al., 2001; Mano et al., 2004; Zhao et al., 2008).

Bonfield and colleagues firstly conceived hydroxyapatite-reinforced polymer biocomposites as a bone analog biomaterials enabling their properties to be tailored to mimic those of bone tissues (Bonfield et al., 1981; Bonfield, 1988a; Bonfield, 1988b; Di Silvio et al., 2002). However, biological apatites are non-stoichiometric hydroxyapatite (HA) as traces of ions are found in HA lattice (Barralet et al., 1998; Kovaleva et al., 2008). Among them, carbonate (CO_3^{2-}) is the most abundant species, ranging from 2 to 8 wt%, depending on the individual's age (Rey et al., 1989; Krajewski et al., 2005; Kovaleva et al., 2008). B-type CO_3^{2-} substitution which involves substitution of CO_3^{2-} at PO_4^{3-} site has higher affinity for human trabecular osteoblastic cell and is dominant in young tissues (Landi et al., 2000; Freiman and Cook, 2007). The dissolution rate of HA improves with CO_3^{2-} doping, which, in turn,

enhances its osteointegration rate (Hasegawa et al., 2003; Morales-Nieto et al., 2013).

Of all the biodegradable polymers which show promise in biomedical field, poly(L-lactide) (PLLA) is the most prevalent. Not only it has good mechanical properties, it degrades into lactic acids which subsequently be eliminated as carbon dioxide (CO_2) and water via Krebs cycle (Lasprilla et al., 2011). Therefore, it has been approved by U.S. Food and Drug Administration (FDA) to be used for a number of clinical applications (Engineer et al., 2011). Addition of HA into PLLA not only buffers the acidic resorption of PLLA, it also helps to improve its mechanical properties and enhances cell proliferation and alkaline phosphatise activity (Hutmacher, 2000; Wei and Ma, 2004; Duan et al., 2010; Wang et al., 2010).

Another biodegradable polymer which has been frequently studied and applied in several biomedical applications is poly(vinyl alcohol) (PVA). It is relatively low cost biodegradable polymer with good biocompatibility, bioinert and hydrophilic properties. Thus, PVA is normally studied on its potential use as drug carrier in hydrogel form, as template for in situ precipitation of nano-sized HAp particles in the fabrication of a porous HA/gelatin composite (Paradossi et al., 2003; Sinha et al., 2003; Nayar and Sinha, 2004; Lasprilla et al., 2011).

1.2 Problem Statement

Typically, HA or carbonated hydroxyapatite (CHA) powders are incorporated into biodegradable polymers (PLLA and PVA) as filler rather than vice versa, i.e. as matrix (Shikinami and Okuno, 1999; Komlev et al., 2003; Hong et al., 2005, Yusong

et al., 2007; Rogel et al., 2008). However, researchers have found that acidic degradation of the polymers would attribute to the pH drop *in-vitro* or *in-vivo* and thus cause undesired environment for cells such as inflammatory during implantation (Schiller and Epple, 2003; Rezwan et al., 2006).

Increasing amount of HA was found to not only buffer the acidic resorption, it also improved the properties of biocomposites in term of mechanical properties and bioactivity, which is a very important characteristic for bone substitution applications (Hutmacher, 2000; Wang et al., 2010). However, biological apatites are predominantly B-CHA which have higher affinity for human trabecular osteoblastic cells as compared to HA and A-CHA (substitution of CO_3^{2-} at OH^- sites) (Rey et al., 1989; Landi et al., 2000; Freiman and Cook, 2007). Zhou et al. (2008) had successfully synthesized nanosized B-CHA by nanoemulsion without any potentially toxic surfactant addition. This method provides reproducible narrow size distribution nanoparticles as well (Solans et al., 2005; Zhou et al., 2008). On other hand, porosity is very essential aspect for vascularization and growth of bone into an implant. Nevertheless, it can always be introduced by the slow removal of materials (Roeder et al., 2008).

Therefore, in this study, biocomposites of B-type CHA with different loadings of PLLA and PVA were fabricated and investigated, with CHA as the matrix. PVA, due to its hydrophilicity, was hypothesized to be removed from the biocomposite first, resulting in higher porosity and providing higher surface area for improved dissolution and precipitation rate to induce apatite growth. PLLA, on the

other hand, was expected to slowly degrade along with CHA, while providing improved mechanical properties for the biocomposites.

1.3 Objective of the Research

The aim of this research is to produce CHA/PLLA/PVA biocomposite with adequate strength, good physical properties, and biocompatibility. With this main objective, the following studies were conducted:

i. To synthesize B-type CHA powder via nanoemulsion by two techniques, i.e. dropwise and direct pouring method, to ascertain the most effective method.
ii. To determine the optimum annealing temperature for CHA which retain adequate B-type carbonate content.
iii. To prepare CHA/PLLA/PVA biocomposite in different ratios and investigate its physical, chemical, morphological and mechanical properties.
iv. To evaluate the bioactivity of CHA/PLLA/PVA biocomposite via simulated body fluid (SBF) solution.

1.4 Scope of Work

This study is comprised of three stages of research work, starting with synthesis of carbonated hydroxyapatite (CHA), followed by the study of effect of annealing on the as-synthesized CHA powder (300–900 °C), and lastly the blends of CHA powder with biopolymers i.e. PLLA and PVA.

In the synthesis of CHA powder, two synthesis techniques, namely dropwise (DW) and direct pouring (DP), were applied in nanoemulsion method and compared.

The better synthesis technique was then carried out on the study of addition of various carbonate content on the properties of as-synthesized CHA powder.

The optimized as-synthesized CHA powder was selected to investigate the influence of different annealing temperatures on the powder. Carbonation was employed at the end of annealing process in order to compensate the loss of carbonate content during the heat treatment.

As for the final stage, biocomposites of optimized annealed CHA powder with different loading of PVA and PLLA were fabricated by powder compaction and heat treated.

Different characterization techniques were undergone to study the physical, chemical, thermal and mechanical properties of CHA powder and its biocomposites. The bioactivity of the biocomposites was also investigated.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

In this chapter, review on bone is firstly introduced, followed by different type of bone graft substitutions (BGS), whereby the pros and cons of each method are compared. The advantages of carbonated hydroxyapatite (CHA) over stoichiometric hydroxyapatite (HA) under category bioceramics will be provided, and as well as the synthesis of CHA by nanoemulsion method. Then, the properties and production method of the two of the biodegradable polymers applied in this study, i.e. poly(L-lactide) (PLLA) and poly(vinyl alcohol) (PVA), are reviewed. Lastly, biocomposites of HA/PLLA, CHA/PLLA, and HA/PVA are presented and discussed.

2.2 Natural Human Bone

Bone is a substantial unit of human skeleton system, which acts as a support for the body and its movements (Wang, 2004), together with muscles, ligaments, and tendons (Park and Lakes, 2007). The hierarchy of human bone has three levels. First is the macrostructural level, which is divided into trabecular and compact bone. This is followed by a microstructural level, ranging 1-500 μm, which includes lamellae, cells, osteons and Harvesian systems. Lastly, at nanostructural level, the smallest structure in bone which includes non-collagenous proteins, fibrillar collagens and embedded hydroxyapatite crystals (50 x 25 x 3 nm^3) (Chen and Webster, 2011). Figure 2.1 depicts the hierarchical organization of a bone structure.

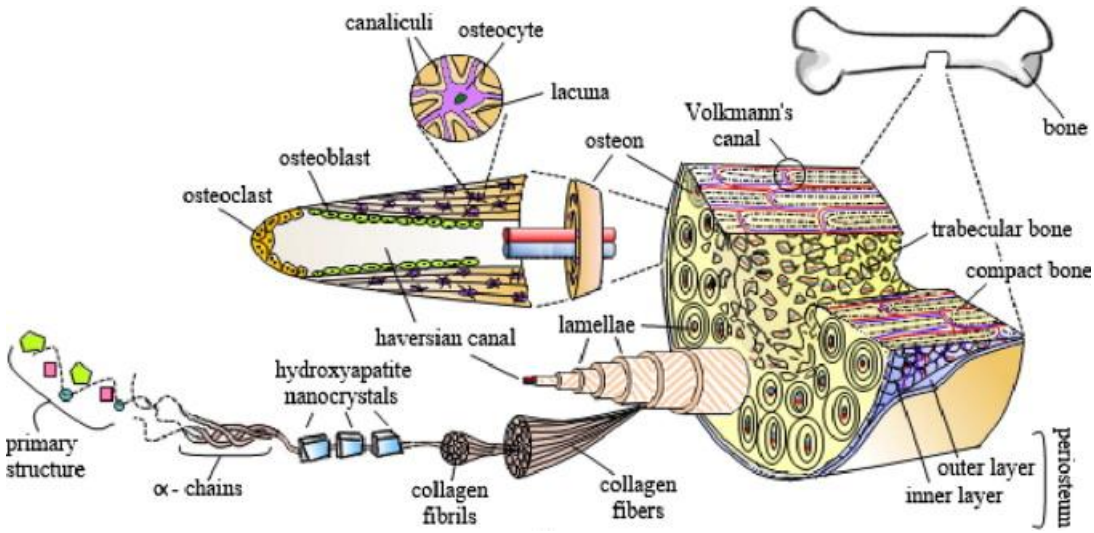

Figure 2.1: The general structure of bone, showing its hierarchical organization (Luz and Mano, 2010)

Lakes (1993) stated that at different scales, bone could be fibrous, laminar, particulate and porous. Apart from that, it contains bone-forming cells (osteoblasts) and bone-resorbing cells (osteoclasts) and various osteoconductive growth factors and molecules. In most bones, the major component of human bone is mineral substances (60-70 wt%), 20-30 wt% is of collagen and other organic components, while remaining is water (Weiner and Zaslansky, 2004; Currey, 2008). Table 2.1 summarizes the comparison in chemical composition, crystal structure, and other properties of adult human enamel, dentine and bone.

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Table 2.1: Composition and physical properties of apatites in adult human enamel, dentine, and bone (Park, 2008)

Composition	Enamel	Dentine	Bone
Calcium, Ca^{2+}	36.5	35.1	34.1
Phosphorus, P	17.7	16.9	15.2
(Ca/P) molar	1.63	1.61	1.71
Sodium, Na^{+}	0.5	0.6	0.9
Magnesium, Mg^{2+}	0.44	1.23	0.72
Potassium K^{+}	0.010	0.05	0.03
Carbonate, CO_3^{2-}	3.5	5.6	7.4
Fluoride, F^{-}	0.01	0.06	0.03
Chloride, Cl^{-}	0.30	0.01	0.13
Pyrophosphate, $P_3O_7^{4-}$	0.022	0.10	0.07
Total inorganic (mineral)	97.0	70.0	65.0
Total organic	1.5	20.0	25.0
Absorbed H_2O	1.5	10.0	10.0
Traces elements: Sr^{2+}, Pb^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}, etc.			
Crystallographic properties			
Lattice parameters (+0.0003 nm)			
a-axis	0.9441	0.9421	0.941
c-axis	0.610100	0.610107	0.6109
"Crystallinity index"	70~75	33~37	33~37
Crystallite size (nm)	0.13 x 0.03	0.020 x 0.004	0.025 x 0.003

2.2.1 Compact Bone

The compact bone, also known as cortical bone, is dense with porosity in the range of 5-10%. It is normally found in the shafts of long bone (femur, tibia, fibula) (Currey, 1998) and outer layers of flat bones such as skulls and mandibles (Brandi, 2009). Having four times higher mass and significant smaller surface area as compared to trabecular bone, compact bone is responsible for 80% of skeletal mass (Brandi, 2009), with density of 1.99 g/cm^3 (Currey, 1998).

In terms of mechanical properties, it exhibits high strength and stiffness when load is applied at longitudinal direction (Currey, 1998; Bartel et al., 2006). All the above advantages render it to act as a support to body weight and to protect internal organ (Doll, 2005; Behari, 2009).

2.2.2 Trabecular Bone

Also referred as cancellous or spongy bone, the trabecular bone is less dense, with a porosity of 50-90% (Buckwalter et al., 1996). This bone can be found across the ends of long bones and inner parts of flat bones (Brandi, 2009). Its cross section appears like honeycomb and is composed of short struts of bone material called trabeculae (Hench and Wilson, 1993). The intertrabecular spaces of the highly porous trabecular bone are filled with blood vessels and cells (Behari, 2009).

The mechanical properties of trabecular bone depend on the porosity of specimen and architectural arrangement of the individual trabeculae (Black and Hastings, 1998). With its finer structure and greater surface area than compact bone, it provides maximum support by minimum bone material. When calcium and phosphorous in body are insufficient, trabecular bone with higher surface area will be resorbed first to maintain equilibrium within the body (Doll, 2005; Behari, 2009). Table 2.2 compares the structural and mechanical properties of both compact and trabecular bone.

Table 2.2: Comparison between structural features and mechanical properties of compact and trabecular bone (Hench and Wilson, 1993; Keaveny 1998)

Properties	Cortical bone	Trabecular bone
Volume fraction	0.85-0.95	0.05-0.60
Surface/bone volume (mm^2/mm^3)	2.5	20
Total bone volume (mm^3)	1.4×10^6	0.35×10^6
Total internal surface (mm^2)	3.5×10^6	7.0×10^6
Compressive strength (MPa)	100-230	2-12
Flexural strength (MPa)	50-150	10-30
Strain to failure	1-3	5-7
Young modulus (GPa)	7-30	0.1-2.0

2.2.3 Bone Graft Substitute (BGS)

Bone grafting is a transplantation of bone to a patient by surgical operation, in order to repair or replace the damaged or diseased bones. Ideally, remodelling of bone should happen as the implant promotes new bone growth, reinforce the repaired area and in time, being replaced by the newly formed bone (Hing, 2005). Generally, there are four types of bone graft, namely autograft, allograft, xenograft, and synthetic graft.

2.2.3.1 Autograft

Autograft is obtained through bone transplantation from other parts of the patient himself. Being referred as the "gold standard", this autogenous bone grafting offers the most biocompatible bone substitution, as the body accepts its own component better than from a donor's (LeGeros, 2008). Besides, there is no risk of disease transmission and will not cause any immune rejection to the patient after implantation (Hing, 2005).

This method, however, has several disadvantages which are needed to be taken into serious consideration. Firstly, it requires the patient to suffer another surgical operation at different parts of the body, creates another defect area at harvest site, and has limited availability (Mata et al., 2002; LeGeros, 2008). This autografting is thus time-consuming and the chance of morbidity at harvest site is increased. Another challenging part is to obtain desired shape and size according to damaged area as it is hard to substitute perfectly. Another concern is the different resorption rate between the substituted and surrounding bones (Baino, 2011).

2.2.3.2 Allograft

Allografting, also known as allogeneic bone grafting or homografting, is the transplantation of bone from another living patient or cadaver to the patient (Bohner, 2010). In modern allografting, bone used is stored within regulated bone banks and freeze-drying is generally used to decrease its antigenicity and extend the storage period (Sutherland and Bostrom, 2003). This technique has solved some drawbacks of autografting such as lack of donor bone site from own body, twice surgical operations and possible morbidity. The surgical time can thus be reduced, and pre-forming of shape and size of transplant bone can be done.

Nonetheless, allografting has concerns which include tissue availability, graft cost, delayed graft incorporation (body fails to accept foreign tissue), sterilization, and long term graft strength as well as creating pain to donor. Harvesting bone from living people or corpse has possible disease transmission or virus infection from donor. Careful checking procedure must be therefore applied, resulting in time-consuming and cost increment of this transplant (Sutherland and Bostrom, 2003).

Incompatible blood group between donor and patient can induce development of antibodies within ABO blood group system of patient (Schnettler et al., 2006).

2.2.3.3 Xenograft

By transplanting of living cells, tissues or organs from other mammalian species (normally cows or pigs) to human, xenografting solves the scarcity of donor site. However, xenograft is not as biocompatible as autograft, and like allograft, as there are risks of disease and virus transmission from animal to patient and hyperacute rejection. Different aging rate of xenograft and human is a concern for this method as well (Platt et al., 1990). Moreover, perceived ethical disadvantages and religious beliefs (Muslim, Jewish) which prohibit the use of pigs as source also hinder the use of xenografting (Boneva et al., 2001; Sopyan and Algap, 2009).

2.2.3.4 Synthetic Graft

Synthetic grafts refer to the synthetic materials used as bone implant materials. It is widely used instead of the other grafting methods as it solves most of the disadvantages faced by those methods. They are mostly from family group of calcium phosphate (CaP), especially HA-based materials. Its shape and size can be tailored to perfect fitting, while its composition and resorption rate can be controlled to produce biocompatible and biodegradable synthetic materials. Composites of alloplastic materials can be produced to complement each material's shortcoming and give attractive properties as bone substitutes. The utilization of biodegradable alloplastic graft eliminates the need of second surgical operation. Problems such as disease transmission, morbidity and lack of bone donor site will not occur with this method (Bohner, 2010; Baino, 2011).

There is still a probability that patient's body will reject CaP-based synthetic graft even though it is biocompatible, while the synthetic graft currently do not have exactly same mechanical properties as natural bone. There is potential wear on the implanted graft especially as joint replacement (Baino, 2011).

2.3 Biomaterials

Generally, biomaterial is defined as a synthetic material used to replace part of a living system or to function in intimate contact with living tissue. It substitutes the diseased or traumatic body part and assists in healing, improves function and corrects abnormalities (Park, 1995).

The fundamental requirement of a biomaterial is its ability to perform effectively with an appropriate host response in a specific application, which can be termed as 'biocompatibility'. There are three important aspects of biocompatibility that a candidate biomaterial seeks to achieve:

i. biochemically compatible, non-toxic, non-irritable, non-allergenic and non-carcinogenic,
ii. biomechanically compatible with surrounding tissues, and
iii. bioadhesive contact must be established between the materials and living tissues.

During the selection of a biomaterial, its biological properties and responses in physiological environment are more emphasized as compared to its superior mechanical properties (Basu and Nath, 2009). Biomaterials can be widely categorized under four categories: polymers, metals, ceramics and composites, which

are tabulated in Table 2.3. The advantages, disadvantages and examples of each category of biomaterials are also presented.

Table 2.3: Class of materials used in the body (Park, 1995; Park and Lakes, 2007)

Materials	Advantages	Disadvantages	Examples
Polymers (nylon, silicone, rubber, polyester, etc)	• Resilient • Easy to fabricate	• Not strong • Deforms with time • May degrade	Sutures, blood vessels, hip socket, ear, nose
Metals (Ti and its alloys, Co-Cr alloys, Au, Ag stainless steels, etc.)	• Strong • Tough • Ductile	• May corrode • Dense • Difficult to make	Joint replacements, dental root implants, pacer and suture wires, bone plates and screws
Ceramics (alumina zirconia, CaP including HA, carbon)	• Very biocompatible • Inert • Strong in compression	• Brittle • Not resilient • Weak in tension	Dental and orthopaedic implants
Composites (carbon-carbon, wire- or fiber-reinforced bone cement)	• Strong • Tailor-made	• Difficult to make	Joint implants, heart valves, bone cement, dental resin

2.4 Bioceramics

Ceramics that are used to repair and replace diseased and damaged parts of human musculoskeletal systems are referred to as bioceramics (Thamaraiselvi and Rajeswari, 2004; Arcos and Vallet-Regí, 2013). These bioceramics are highly biocompatible as compared to polymeric or metallic biomaterials, and are often used as implants within bones, joints and teeth in bulk. Nonetheless, bioceramics are

limited to compressive load application due to its brittleness and very low tensile strength (TS) (Billotte, 2003; Dorozhkin, 2010a).

The implantable bioceramics must be non-toxic, non-carcinogenic, non-allergic, non-inflammatory, biocompatible, and biofunctional for its lifetime in the host (Bajpai and Billotte, 1995). Ceramics used in fabricating implants can be categorized as nonresorbable (relatively inert), bioactive or surface reactive (semi-inert), and biodegradable or resorbable (non-inert), as displayed in Table 2.4.

Table 2.4: Classification of bioceramics by implant-host response (Bajpai and Billotte, 1995)

Classification	Implant-host response	Examples
Relatively bioinert or nonresorbable	• Maintain physical and mechanical properties while in the host • Resist corrosion and wear	Carbon, dense and porous alumina (Al_2O_3), zirconia (ZrO_2), dense HA
Bioactive or surface-reactive	• Form strong bonds with adjacent tissue upon implantation in host	Bioglasses and ceravital, dense glasses, HA
Biodegradable or resorbable	• Degrade on implantation in host • Replaced by endogenous tissues	Glass fibres and their composites, corals, HA, tricalcium phosphate (TCP)

2.4.1 Hydroxyapatite

Ceramics and composites based on calcium phosphates are known for their applications as bone implants to substitute damaged or diseased bones. Amongst them, the stoichiometric hydroxyapatite (HA) with a chemical formula of $Ca_{10}(PO_4)_6(OH)_2$ has received wide attention for the past decades for its affinity to bone minerals and exhibits biocompatibility, bioactivity and osteoconductivity

(Suchanek et al., 2002; Murugan and Ramakrishna, 2006; Lafon et al., 2008; Chang et al., 2011).

HA is a member of the apatite group of ceramics. Apatite describes a family of compounds having hexagonal system with space group of $P6_3/m$ irrespective of different composition (LeGeros et al., 2009). The stoichiometric HA has Ca/P ratio of 1.67, and as reported in ICDD 9-432, has unit cell dimensions of $a = b = 9.418$ Å and $c = 6.884$ Å, and calculated density of 3.16 g/cm^3. HA structure is depicted in Figure 2.2.

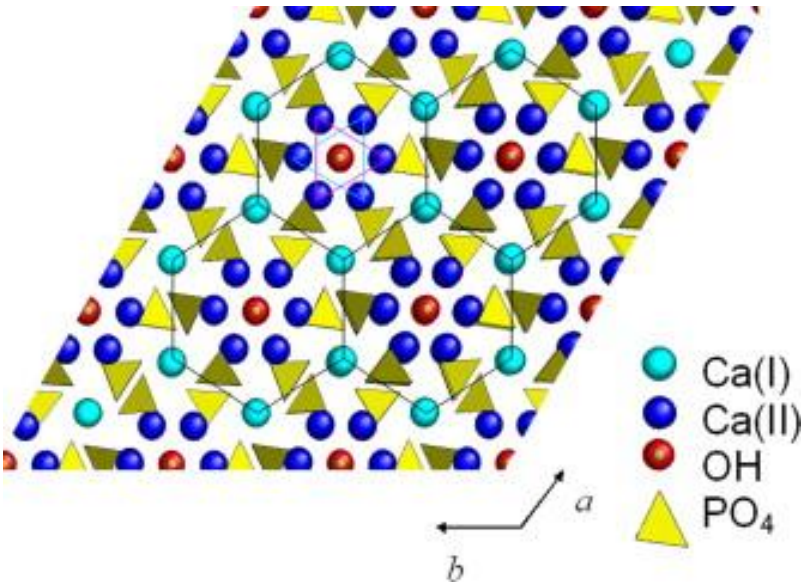

Figure 2.2: The HA structure viewed along the c-axis. Black lines connect Ca(I) columns in hexagonal networks, while cyan and magenta triangles connect staggered Ca(II) atoms lying in the same plane but at different height with respect to the c-axis (Boanini et al., 2010).

The crystal structure of HA is very "hospitable", allowing substitutions of many other ions such as F^-, Cl^-, CO_3^{2-}, Na^+, and Ba^{2+}, for Ca^{2+}, PO_4^{3-}, or OH^- groups.

The ionic substitutions will result in changes of properties (crystallography, lattice parameters, morphology, solubility, thermal stability, etc.) (LeGeros et al., 2009).

Synthetic HA has been produced in granules, blocks, scaffolds, as composites with polymers or other ceramics, or as coatings in biomedical application. Despite its ability to have direct chemical bonding with bone, it has low solubility and will not resorb *in vivo*. As compared to biogenic apatite, HA's resorption is too sluggish to induce massive formation of new bone tissues (Ishikawa et al., 2003; Kovaleva et al., 2008).

However, HA has inadequate mechanical strength especially in aqueous environment, hindering its usage as load-bearing implants. As reported by Jarcho (1981), the Weibull factor of dense HA is 12 in wet physiological environment, which means that it may fail by fatigue within several months of application. Dense HA is very brittle, with Young's modulus ranging from 40-117 GPa, which is appreciably higher than living bone's (Park, 2008). Hence, the application HA is limited to non-load bearing prostheses, including bone filler and scaffolds for bone reconstruction. The mechanical properties of HA are summarized in Table 2.5.

Table 2.5: Mechanical properties of synthetic HA (Park, 2008)

Properties	Values
Young's modulus (GPa)	40-117
Compressive strength (MPa)	294
Bending strength (MPa)	147
Hardness (Vickers, GPa)	3.43

2.4.2 Carbonated Hydroxyapatite

Biological apatites have been reported to be nanosized with low crystallinity and contain traces of ions substituted in HA lattice, where carbonate (CO_3^{2-}) is the most abundant species (Barralet et al., 1998; Gibson and Bonfield, 2002; Vallet-Regí and González-Calbet, 2004; Kovaleva et al., 2008). Hence, biological apatites are typically also referred as carbonated hydroxyapatite (CHA) (Merry et al., 1998; Heslop et al., 2005; Leventouri, 2006).

The carbonate content ranges from 2 to 8%, depending on the individual's age (Rey et al., 1989; Bigi et al., 1997; Krajewski et al., 2005). HA may be chemically doped with small amount of CO_3^{2-} (up to 20 mol%), whereby the incorporation has considerable impact on the stability of crystal lattice of HA (Best et al., 2008). The dissolution rate improves with CO_3^{2-} doping, in which, the local concentrations of Ca^{2+} and PO_4^{3-} ions that are necessary for new bone formation are increased. Therefore, as compared to stoichiometric HA, CHA shows better bioresorbability in physiological fluid and enhanced osteointegration rate (Doi et al., 1999; Hasegawa et al., 2003; Morales-Nieto et al., 2013). Moreover, the partial substitution of CO_3^{2-} tends to decrease the crystallinity of HA which is closer to mimicking the biological bone (He et al., 2008). It is always desirable that a bone substitute be bioresorbable to some extent so that over a period of time, it will be replaced with the regenerated bone (Murugan and Ramakrishna, 2006).

Possessing good bioactivity, biocompatibility and osteoconductivity properties, synthetic CHA has attracted much attention in tissue engineering (TE) field, bone graft substitution as well as dental replacement and reparation.

Nonetheless, the mechanical properties of apatite ceramics are less than that of the human cortical bone, which limits the application of CHA to non-structural implants (Nakamura, 1996).

2.4.2.1 Classification of Carbonated Hydroxyapatite

Classification of CHA depends on the mode of CO_3^{2-} substitution in HA, giving A-type CHA (CO_3^{2-} substitutes OH^-), B-type CHA (CO_3^{2-} substitutes PO_4^{3-}) or AB-type CHA (CO_3^{2-} substitutes both OH^- and PO_4^{3-}) (Vignoles et al., 1988; Gibson and Bonfield, 2002; Lafon et al., 2008). Biological apatites are predominantly B-CHA with A/B type ratio of 0.7-0.9 (Rey et al., 1989). Higher A/B type ratio was observed in old tissues as compared to the young ones, indicating that type B carbonate apatite is dominant in young tissues (Landi et al., 2004).

Synthesis method and condition have been reported to affect the type and properties of CHA produced. A-CHA, $Ca_{10}(PO_4)_6(OH)_{2-2x}(CO_3)_x$ with $0 \leq x \leq 1$, is commonly synthesized by heating HA at high temperature (800-1000 °C) in dry CO_2 atmosphere for a few hours, where the reaction is presented in Eq. 2.1 (Gibson and Bonfield, 2002; Lafon et al., 2008). Each CO_3^{2-} group substitutes for two OH^- group so the charge balance is maintained.

$$Ca_{10}(PO_4)_6(OH)_2 + xCO_2 \leftrightarrow Ca_{10}(PO_4)_6(OH)_{2-2x}(CO_3)_x + xH_2O \qquad \text{(Eq. 2.1)}$$

On the other hand, B-CHA are normally obtained from precipitation reaction in aqueous media, which gives rise to the chemical formula of $Ca_{10-y}(PO_4)_{6-y}(CO_3)_y(OH)_{2-y}$ with $0 \leq y \leq 2$ (Lafon et al., 2008). The charge imbalance of the

produced B-CHA can be balanced by introducing sodium carbonate (Na_2CO_3), as reported by Gibson and Bonfield (2002), as shown in Eq. 2.2. In order to mimic the natural structure, sodium salts are often used in syntheses, as apatite structure of the mineral bone contains Na^+ ions which are located in the calcium sites (Lafon et al., 2008).

$$Ca_{10-y}Na_y(PO_4)_{6-y}(CO_3)_y(OH)_2 \text{ or } Na^+ + CO_3^{2-} \leftrightarrow Ca^{2+} + PO_4^{3-} \qquad \text{(Eq. 2.2)}$$

The CO_3^{2-} substitution in HA crystal structure is known to alter Ca:P molar ratio (Barralet et al., 2002) as well as its crystal lattice properties, due to the size difference between PO_4^{3-}, OH^-, and CO_3^{2-} ions (LeGeros et al., 1969). The comparisons between A- and B-type CHA are tabulated in Table 2.6. B-type CHA, with lower A/B ratio, is more preferred as compared to A-type as it dissolves without changing surface polar property in bioactivity test (Landi et al., 2004).

With a combination of wet chemical reaction followed by sintering under CO_2 gas atmosphere, AB-type CHA can be yielded with the chemical formula of $Ca_{10-y}(PO_4)_{6-y}(CO_3)_y(OH)_{2-y-2x}(CO_3)_x$ with $0 \leq y \leq 2$ and $0 \leq x \leq y/2$, where x and y correspond to the A-type and B-type carbonate contents, respectively (Gibson and Bonfield, 2002; Lafon et al., 2008).

Table 2.6: Comparison of A- and B-type CHA (LeGeros et al., 1969; LeGeros, 1991; Ito et al., 1997; Redey et al., 1999; Landi et al., 2004; Slosarczyk et al., 2005; Kovaleva et al., 2008; Zyman and Tkachenko, 2011)

Properties	A-type CHA	B-type CHA
CO_3^{2-} substitution	OH^- site	PO_4^{3-} site
A/B ratio	Higher	Lower
Most abundant species	Old tissues	Young tissues
Preparation	Heating in dry CO_2 atmosphere	Synthesized by wet and dry chemical methods
Synthesis temperature	High (> 800 °C)	Low
Order of solubility increment after incorporation of one CO_3^{2-} per unit cell in HA	$10^{15.9}$	$10^{11.0}$
Unit cell parameters		
• *a*-axis (Å)	Expansion (> 9.418)	Contraction (< 9.418)
• *c*-axis (Å)	Contraction (< 6.884)	Expansion (> 6.884)
• *c*/*a* ratio	Lower (< 0.73)	Higher (> 0.73)
Miller's plane		
• (300)	Shift to lower angle	Shift to higher angle
• (002)	Shift to higher angle	Shift to lower angle
Infrared spectrum (CO_3^{2-} bands)	877-880, 1500, 1540-1545 cm^{-1}	870-875, 1410-1430, 1450-1470 cm^{-1}
Chemical formula	$Ca_{10}(PO_4)_6(OH)_{2-2x}(CO_3)_x$ with $0 \leq x \leq 1$	$Ca_{10-y}(PO_4)_{6-y}(OH)_{2-y}(CO_3)_y$ with $0 \leq y \leq 2$
Preference	Less preferable, lower affinity for human trabecular osteoblastic cell as compared to HA.	Preferable, decrease in crystallinity and increase in solubility without changing surface polar property in both *in vivo* and *in vitro* tests.

2.4.2.2 Synthesis of Carbonated Hydroxyapatite by Nanoemulsion

Parameters such as pH, temperature, chemical nature and concentration of starting materials control the chemical properties of the final powder (Vignoles et al., 1988). Various synthesis methods used to produce nanocrystalline CHA include microwave irradiation (Sampath Kumar et al., 2000), mechanochemical (Suchanek et al., 2002), hydrothermal (Riman et al., 2002; Jokanović et al., 2006), mechanochemical-hydrothermal (Riman et al., 2002; Suchanek et al., 2002), precipitation (Lafon et al., 2008), nanoemulsion (Zhou et al., 2008), and hydrolysis (Pieters et al., 2010).

Nanostructured CHA particles have high surface area which is desirable for their use in many fields (including TE) as to imitate bones and teeth's architectures. Zhou et al. (2008) succeeded in synthesizing nanosized B-CHA by adopting nanoemulsion method without addition of any surfactant, and the results suggested it was calcium deficient with Ca/P molar ratio less than 1.67.

Basically, emulsion is a mixture of at least two immiscible liquids in which one liquid (the dispersed phase) is dispersed in the other (the continuous phase). It is classified either as water-in-oil (W/O) or oil-in-water (O/W), depending on which phase constitutes the dispersed phase (Forgiarini et al., 2001). Emulsion is a thermodynamically unstable liquid/liquid dispersion that is normally stabilized by surfactants (typically over 20 wt%). Energy inputs such as shaking, stirring, homogenizing or spraying are needed to form an emulsion, and the preparing process is termed emulsification.

Nanoemulsion, also known as submicron emulsion, consists of fine and uniform droplets ranging from 20-500 nm (Solans et al., 2005; Zhou et al., 2008). Unlike macroemulsion and microemulsion, nanoemulsion is formed spontaneously, where two immiscible fluids with low interfacial tension are brought into contact. The principle of this method is that preformed material can precipitate as nanospheres when the organic phase (solution water in miscible organic solvent) is mixed with aqueous phase (water containing surfactants) (Zhou et al., 2008). In biomaterials development where biocompatibility is a crucial requirement, this method eliminates the concern of potentially toxic surfactants as lower concentration or no surfactants are needed while providing reproducible narrow size distributed nanoparticles.

Acetone is one of the common solvents for $Ca(NO_3)_2 \cdot 4H_2O$, apart from ethanol (EtOH). Cheng et al. (2001) and Liu et al. (2001) had applied ethanol in their research of hydroxyapatite (HA) syntheses via sol-gel production route. However, it was reported that EtOH is a polar protic solvent, whereby when dissolved with $Ca(NO_3)_2 \cdot 4H_2O$, some nitrate groups would be substituted by ethoxide groups and resulted the formation of $Ca(OEt)_x(NO_3)_{2-x}$ which could later be incorporated into the apatite structure. Acetone, known as polar aprotic solvent (i.e. dissolves without any reaction), was chosen by Zhou et al. (2008) as oil phase in the nanoemulsion synthesis, which do not affect the purity of the as-synthesized CHA powder. Besides, it exists in normal blood and urine as product of breakdown of body fat in very little quantities and its selection as reaction medium mimics the natural process of apatite formation (Reisman, 1998; Zhou et al., 2008).