CHARACTERIZATION AND PROPERTIES OF SYNTHESIZED AND SINTERED CARBONATED HYDROXYAPATITE

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Master of Science

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DECLARATION

I hereby declare that I have conducted, completed the research work and written the dissertation entitled "Characterization and Properties of Synthesized and Sintered Carbonated Hydroxyapatite". I also declare that it has not been previously submitted for the award for any degree or diploma or other similar title for any other examining body or University.

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YANNY MARLIANA BT BABA ISMAIL

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

BET	Brunauer, Emmet and Teller
BGS	Bone-Graft Substitute
CHA	Carbonated Hydroxyapatite
CHN	Carbon,Hydrogen, and Nitrogen
CO_2	Carbon Dioxide
DP	Direct Pouring
DTS	Diametral Tensile Strength
DW	Dropwise
FESEM	Field Emission Scanning Electron Microscope
FTIR	Fourier Transform Infra-Red
FWHM	Full Width at Half Maximum
HA	Hydroxyapatite
ICSD	International Centre of Standard Data
rpm	revolutions per minute
SBF	Simulated Body Fluid
S.G	Specific Gravity
TEM	Transmission Electron Microscope
XRD	X-Ray Diffraction
XRD	X-Ray Fluorescence
β-TCP	Beta Tri-Calcium Phosphate
i.e.	That is
e.g.	For example

LIST OF SYMBOLS

α	Alpha
β	Beta
°	Degree
$\% D_s$	Percentage of shrinkage in diameter
$\% t_s$	Percentage of shrinkage in thickness
$\%T$	Percentage of Transmittance
K_{1c}	Indentation fracture toughness
θ	Theta

PENCIRIAN DAN SIFAT-SIFAT BAGI HIDROKSIAPATIT TERKARBONAT YANG DISINTESIS DAN DISINTER

ABSTRAK

Hidroksiapatit terkarbonat (CHA) jenis B telah berjaya disintesis melalui kaedah pengemulsian nano melalui teknik titisan (DW) dan tuangan langsung (DP). Kandungan karbonat dalam DP (8.60%) adalah lebih tinggi daripada DW (7.85%). Kedua-dua teknik menghasilkan serbuk bersaiz nano dengan bentuk partikel yang bervariasi. Adalah didapati pada suhu rendah (≤ 40°C), serbuk CHA yang dihasilkan berbentuk sudut, manakala di atas suhu kritikal ini, campuran hablur bersudut dan rod terhasil. Suhu bilik adalah lebih baik kerana ia menghasilkan serbuk bersaiz nano dengan luas permukaan yang terbesar. Pensinteran kemudiannya dijalankan ke atas sampel CHA tulen, CHA+$Mg(OH)_2$, HA, dan HA+$Mg(OH)_2$ kemudian disejuk dengan dan tanpa kehadiran gas CO_2 basah untuk menghasilkan jenis penggantian karbonat yang berbeza di dalam struktur. CHA jenis B berjaya dikekalkan dengan pensinteran CHA dan CHA+$Mg(OH)_2$ pada suhu 700-900°C, dengan CHA+$Mg(OH)_2$ mencapai ketumpatan optimum pada 800°C (CM2C). Sampel lain memerlukan suhu lebih tinggi untuk penumpatan. Kandugan karbonat juga adalah berbeza dengan CM2C yang tertinggi iaitu 4.30 wt%. CHA jenis AB dan A dihasilkan apabila HA+$Mg(OH)_2$ dan HA digunakan sebagai bahan permulaan. Penggunaan $Mg(OH)_2$ berjaya menurunkan suhu pensinteran dan membantu penumpatan sampel melalui Persinteran Fasa Cecair. Penggunaan gas CO_2 basah juga telah berjaya menggantikan karbonat yang terurai daripada sampel semasa persinteran. Nilai kekerasan, K_{1c}, dan DTS bagi sampel paling tumpat CM2C adalah 2.37 GPa, 3.27 MPa.$m^{1/2}$, and 22.95 MPa, dengan kebioaktifan yang lebih baik dengan pembentukan lapisan apatit yang lebih cepat. Oleh yang demikian, sifat CHA tersinter yang lebih baik telah berjaya dihasilkan di dalam kajian ini.

CHARACTERIZATION AND PROPERTIES OF SYNTHESIZED AND SINTERED CARBONATED HYDROXYAPATITE

ABSTRACT

B-type Carbonated Hydroxyapatite (CHA) was successfully synthesized via nanoemulsion method, through dropwise (DW) and direct pouring (DP) techniques. The carbonate content in DP (8.60%) was higher than DW (7.85%). Both techniques produced nano-sized powders with variation in shapes of particles. It was also found that at temperature (≤ 40°C), the CHA powders were angular while above this critical temperature mixture of rods and angular shape was produced. Room temperature synthesis was better as they produced nano-size CHA with larger surface area. Sintering was then performed on pure CHA, CHA+$Mg(OH)_2$, HA and HA+$Mg(OH)_2$, samples which was cooled down with and without wet CO_2 atmosphere to produce different type of carbonate substitution in the structure. Pure B-type CHA was successfully retained when pure CHA and CHA+$Mg(OH)_2$ was sintered at 700-900°C, with the latter reaching optimum densification at temperature of 800°C (CM2C). Other samples required higher temperature for densification. The carbonate content was also different with CM2C having highest 4.30 wt%. AB-type CHA and A-type CHA was formed for densest sintered samples using HA+$Mg(OH)_2$ and HA as starting material. The use of $Mg(OH)_2$ had successfully reduce the sintering temperature and improve the densification of the sintered samples through Liquid-Phase Sintering. The use of wet CO_2 atmosphere was successfull to compensate the carbonate loss during sintering. The hardness, K_{1c}, and DTS values obtained for densest CM2C were 2.37 GPa, 3.27 $MPa.m^{1/2}$, and 22.95 MPa, respectively, and shows a better bioactivity by rapid formation of apatite crystals. Thus, an enhancement of sintered CHA properties was successfully achieved in this study.

CHAPTER 1

INTRODUCTION

1.1 Research Backgrounds

Bones are a dynamic and highly vascularized tissue which continuously remodel itself throughout the lifetime of an individual. Typically the bones assist in locomotion activity, ensure skeletons have sufficient load-bearing capacity, and protect delicate internal organs of the body as reported by Stevens (2008). However, bones are also prone to injury, defects and aging, resulting from many different causes. For example, trauma, infections, tumour and metabolism can result in bone injury which is a major public health problem (Best et al., 2008; Sopyan, 2009). Dental and orthopaedics treatments also require adequate bone supplies and therefore the interest in bone regeneration is increasing. As a result, bone grafting or bone substitute has become critical in orthopaedics surgery (Sutherland & Bostrom, 2003).

Bone graft can be defined as implanted or transplanted bone from another part of the human body or mammal, or any synthetic material to reconstruct the bone defect. Bone graft should provide a good local and systematic compatibility, the capability of being substituted by bone and completely filling any defects (Schnettler et al., 2004). Bone graft source can be adopted from another part of non-load bearing site of the indivual patient's body (autograft), another human donor tissue (allograft), animal's tissue (xenograft) or synthetic biomaterials (artificial bones). However, it is known that, autograft, allograft and xenograft have serious drawbacks to the patients (Hing, 2005; Stevens, 2008). Among others, the drawbacks include possibly infected by diseases, inadequate supplies and severe pain to the donor of the graft.

Worldwide, especially in United States, the need for bone grafting arose as the population age increases. Based from the data collected from hospitals treating hip fractures, both government and private hospitals, more than 300,000 hip and knee replacement surgeries have been performed mostly to senior citizens of the age 65. The number of hip fractures is expected to be 500,000 annually by the year 2040 as reported by The Malaysian Osteoporosis Society (MOS). In Malaysia, the overall incidence of hip fractures has been reported as 90 per 100,000 individuals (Lee & Khir 2007). The fracture rates are highest among the Chinese (160 per 100,000), followed by Indians (150 per 100,000) and Malays (30 per 100,000) based from the race-specific incidence data. Females were reported to be twice frequently affected compared to the male counterparts based from the data collected by the National Statistic Department. Hence,there has been the need to study on the production of artificial bones from biomaterials. Therefore, technological research has moved towards the synthesis of new substituting biomaterials mimicking biological bone tissue (Tampieri et al., 2005).

Hydroxyapatite (HA) is one of the most widely used bioceramic in bone graft substitute, bone tissue engineering and drug delivery system (Suchanek et al., 2002). This is possible due to its biocompatibility, bioactivity, osteoconductivity and non-toxicity properties (Bouyer et al., 2000; Afshar et al., 2003; Rajabi-Zamani et al., 2008). It is also greatly influenced by its similarity in chemical structure with biological apatite, which comprises of the mineral phase of calcified tissue in the enamel, dentin and bone (Murugan & Ramakrishna, 2006). However, stoichiometric HA has been reported to have limited ability to form an interface and its resorption *in vivo* is too sluggish to induce a massive formation of a new bone tissue (Kovaleva et al., 2008). Stoichiometric synthetic HA also does not degrade significantly but rather

remains as a permanent fixture susceptible to long term failure (Ishikawa et al., 2003).

One way to enhance biological properties of HA implant is through the chemical modification of HA. Carbonated hydroxyapatite (CHA) has been reported to be superior to pure HA for bioresorbable implants (Kovaleva et al., 2008). This is because human and animal bone mineral have been shown to contain significant amounts of carbonate (Tadic et al., 2002; Landi et al., 2004; Krajeswari et al., 2005). It is therefore claimed that the biological apatite be referred as carbonated hydroxyapatite (CHA). Generally, the amount of carbonate in CHA is about 2-8 wt% of the calcified tissue and may vary depending on the age factor (Merry et al.,1999; Barralet et al., 2000; Landi et al., 2003). Carbonate ion can substitute either in the hydroxyl groups (A-type) or the phosphate groups (B-type) or it can also simultaneously substitutes both hydroxyl and phosphate groups (AB-type) as reported by Lafon et al. (2008) and Tkachenko and Zyman (2008). The presence of carbonate in the apatite lattice is known to increase chemical reactivity and would probably contribute to the ease of resorption in bony tissue (Vallet-Regi & Gonzalez-Calbet, 2004). The incorporation of carbonate into the host HA would cause an increased in solubility, decrease in crystallinity, change in crystal morphology and better biological activity (Porter et al., 2005). With this, it is currently accepted that CHA is a prospective material for medicine in order to mimic the composition of natural bone (Kovaleva et al.,2008).

In general, there are various synthesis routes that have been explored to synthesize CHA and among others include chemical precipitation, nanoemulsion, and hydrothermal synthesis. A-type CHA is commonly prepared by exposing HA at high temperature under flow of carbon dioxide. On the other hand, B-type CHA is

normally synthesized using wet method from precipitation reaction in aqueous media with control parameters such as pH, temperature, and reagent concentration (Lafon et al., 2008).

Synthetic CHA of excellent bioactivity, biocompatibility and osteoconductivity has already attracted much attention in the field of tissue engineering as a bioresorbable bone graft substitutes as well as for dental replacement and repairation. However, the mechanical properties of these apatite ceramics are known to be less than that of the cortical bone (Nakamura, 1996). Load bearing implants applications are hindered by the low strength and toughness of CHA, especially in wet environment, whereas applications of bulk CHA for non structural implants such as ossicles in ear have no particular difficulties.

1.2 Problem Statement

Typically, heat treatments are widely used either to prepare dense or porous bulks, granules and during plasma spraying of coatings. Basically, the strength of CHA was found to be strongly dependent on the internal porosity and this can be avoided with proper sintering. It can be anticipated that, increasing the density by sintering will decrease the amount of porosity (Morgan et al., 1997). Typically, a high temperature thermal treatment is required to produce densified ceramic part from powders. However, the limited thermal stability of the CHA is a challenge of major importance as reported by Barralet et al. (2000) and Panda et al. (2003). The presence of carbonate ions in HA structure influence the decomposition and sinterability of CHA. Poor control of heat treatment of CHA would result in carbonate loss, leading to partial or total decomposition of the material and hence

would affect the physical and mechanical properties of the synthetic material (Landi et al., 2000).

Therefore, the aim of this work is to produce and sinter dense CHA with good physical and mechanical properties. In this study, the introduction of $Mg(OH)_2$ as sintering aid improve the sintering and thus it is hoped that higher densification and good mechanical properties could be achieve at a much lower sintering temperature in order to avoid decomposition of CHA. The introduction of wet CO_2 is intelligently designed to compensate the carbonate loss due to decomposition during sintering. Besides that, it is also hoped that the CHA formed will be mechanically, biologically, chemically and structurally similar to the mineral phase in bone.

1.3 Objective of the Research

The aim of this reseach is to produce CHA biomaterial with good physical, mechanical and biological properties in order to mimic the natural human bone mineral. With this main goal, the following objectives were set:

I. To synthesize CHA powders by two different techniques, *i.e.* dropwise and direct pouring by nanoemulsion method and characterization of the powder.

II. To synthesize CHA at four different temperatures (RT, 40, 60, and 80°C) and characterize the powder.

III. To study the effect of sintering conditions on CHA with and without the addition sintering aid, on the physical, and mechanical properties of the sintered samples.

IV. To investigate the bioactivity of the sintered samples using Simulated Body Fluid (SBF) solution.

1.4 Scope of works

Generally, this work can be divided into four main parts. Fig.1.1 Flowchart the scope of work involved in this study.

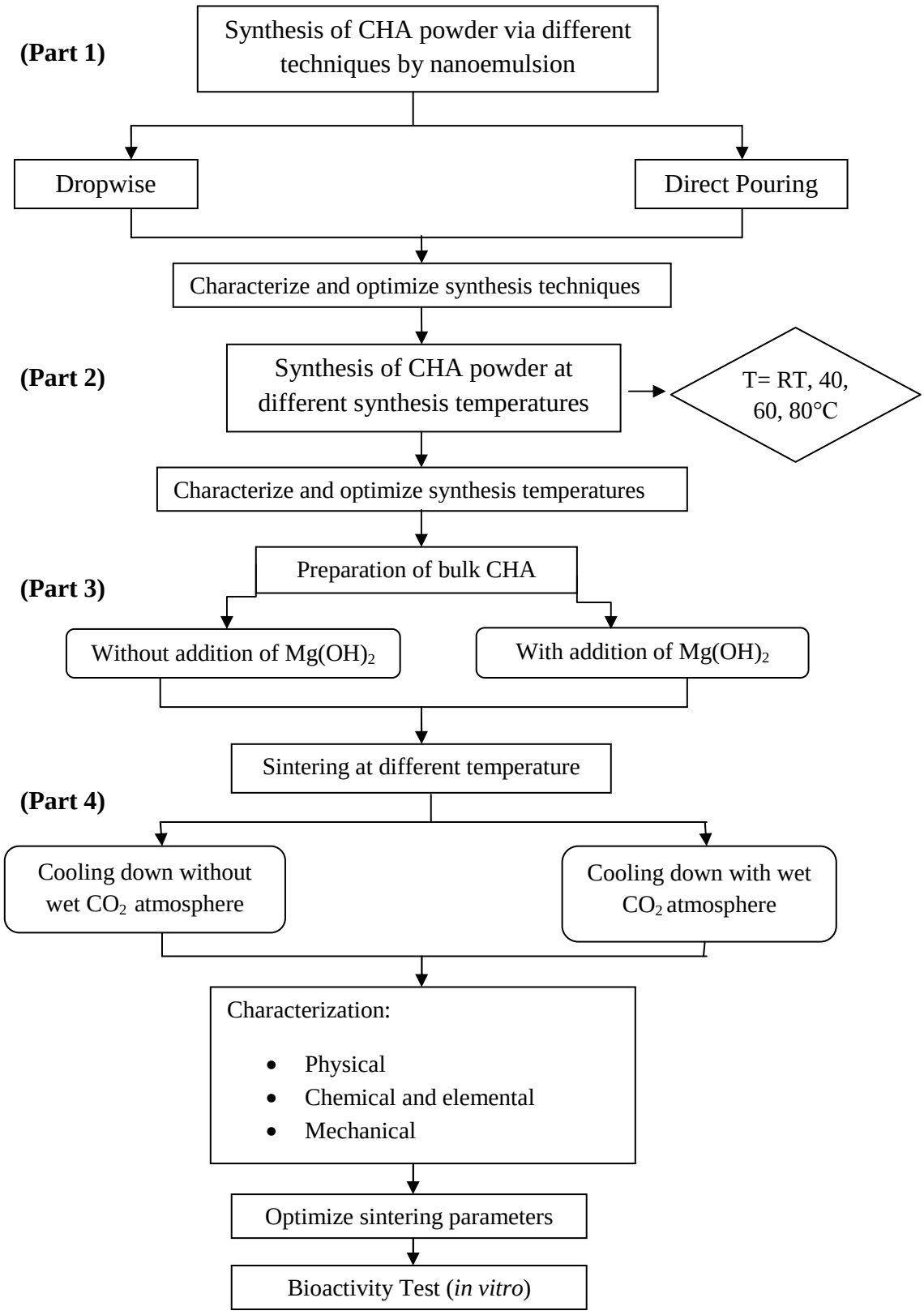

Fig.1.1 Flowchart of the research work

CHAPTER 2

LITERATURE REVIEW

2.0 Introduction

Different reasons could cause injury to bone, and this includes severe missing piece of bone or trauma cases, total hip revisions, infections, cancerous and tumours, metabolism, and in the correction of large "bony defects" involving damage to bone (Sutherland & Bostrom, 2003). Thus, the use of bone substitutes has become clinically important as the demands had increased dramatically over the last few decades. The needs of bone implants arose as the elderly population increased with escalating geriatric health expenditure also in rapidly developing country (Lee et. al, 1993). Worldwide, the total hip fractures in 1990 were reported to be 1.26 million. This was dominated by female that was estimated to be about 917,000 while male was only about 338,000. The number of hip fractures is estimated to be approximately double in the year 2025 which involve 2.6 million patients and 4.5 million by 2050. The major demographic changes will occur in Asia. About 26% of all hip fractures occurred in Asia in 1990 and is estimated to rise to higher number in future. Gullberg et al. (1997) also reported that the socioeconomic impact of hip fractures will increase markedly throughout the world, particularly in Asia. Hence, the needs to develop new substituting artificial bones from biomaterials are getting more important, particularly in developing countries.

Bone grafting was firstly introduced in the 1800's, to replace missing part of the bone with material from either the patient's own body (autograft), or that of a donor (allograft) obtained from a bone bank (Czitrom & Gross, 1992; Meeder & Eggers, 1994). Alternatively, bone grafts can also be adopted from the mammalian's

species as reported by Neumann and Epple (2006). However, the various suggested bone grafts has serious drawbacks to be considered. Allograft, although being considered as the "gold standard" of all bone substitution materials, however have limitations to the amount of collectable bone from donors (Schieker et al., 2006; Ana et al., 2010). Moreover, this method would normally require a second operation which would be costly, time consuming and causes additional trauma (Ishikawa et al., 2003). In the case of allograft and xenograft, there are potentially high risk of transmitting diseases as well as variety of bacterial and virus infections, including those that cause AIDS or hepatitis to patients (Tancred et al., 1998; Schnettler et al., 2004).

Bioceramics are classified as inorganic ceramics that are used in medical and dental practices for the human body (Bilotte, 2003). Ceramics and composite based on calcium phosphate are known to be the potential bioceramic materials for bone graft substitute (BGS). In recent years, synthetic hydroxyapatite (HA) has been extensively use as BGS materials due to its biocompatibility, bioactivity, osteoconductivity and it possess similar characteristic with the biological bone. However, reports showed that human bone differs in composition with stoichiometric synthetic HA in that it contains 2-8 wt% of carbonate ions (Merry et al., 1999; Tadic et al., 2002; Landi et al., 2004; Kovaleva et al., 2008). Hence, there have been drive for researchers to head towards the production of synthetic carbonated hydroxyapatite (CHA), instead of pure synthetic HA, that would be used in BGS application. Additions of other dopants for specific functions have also been investigated.

In general, the aims of this review are to provide a strong fundamental understanding and overview of the structure and properties of natural bone tissues

followed by the important of biomaterial in BGS application. Bioceramic which are a part of biomaterials will be also discussed in detail. The review then will be focussed further on calcium phosphate based bioceramics, in particular the hydroxyapatite and carbonated hydroxyapatite. This will then be followed by the review on the synthesis of CHA via various methods. And the final part of this review then described some available sintering techniques and conditions in order to produce highly densified CHA, as intended in this study.

2.1 Biological Bones

Bone is a typical calcified tissue of mammals; which are in different shapes and sizes from macro- to micro- and to nanoscale, to protect and to provide mechanical support for the body (Currey, 2005; Cui and Ge, 2007). Bone is a complex living tissue, having elegant structure of distinct levels of hierarchical structural units (Stevens & George, 2005; Roveri & Lafisco, 2010) as shown in Fig. 2.1. In simple terms, the bone can be described as a biocomposite of organic phase (based on collagen) in which calcium-containing inorganic crystals are embedded in soft protein matrix (Best et al., 2008, Meyes et al., 2008).

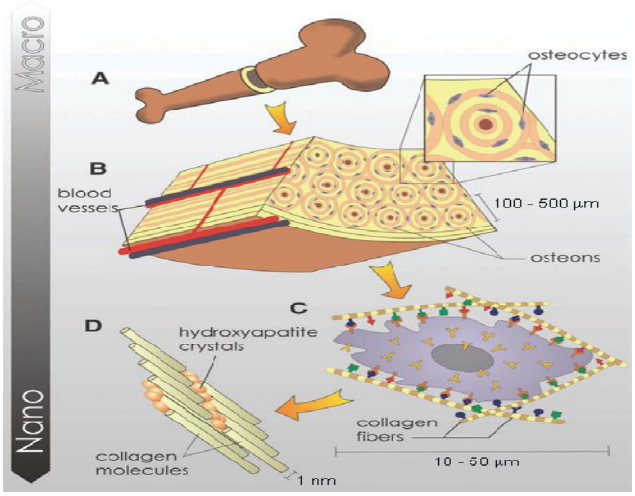

Fig. 2.1 Hierarchical structural units of bone on different scales, including extracellular matrix (Stevens & George, 2005)

The bone structure could be fibrous, laminar, particulate and porous and are present at different scale of size (Dorozhkin, 2009). Though the dimensions of biological apatite crystals reported in the literature varies due to the different treatment methods and analytical techniques, it is generally found that the values in the ranges of 30-50 nm in length, 15-30 nm in width and 2-10 nm in thickness. Nanometer scales appear to be as important to bone as to ensure optimum strength and maximum tolerance of flaws (Gao et al., 2003; Gupta et al., 2006).

From a material engineering point of view, bone is a porous composite material surrounded by bone cells and blood vessels embedded in a biphasic matrix of organic and inorganic elements (Dorozhkin, 2009). Bone also contains bone-forming cells, which are known as osteoblasts and osteoclasts which are the bone-resorbing cells and various osteoinductive growth factors and molecules. Generally, the weight proportions of the major component of human bone are 60- 70% mineral substances, 20-30% of collagen and the other organic components, while the remaining is water (Weiner & Zaslansky, 2004).

Over two decades, hydroxyapatite (HA) has been ascribed as the synthetic material closely resembles bone composition. However, natural bone mineral is relatively an impure version of HA, as it differs in composition from the stoichiometric HA in that it contains additional ions, of which carbonate is the most abundant species (Ishikawa, 2010). The carbonate content varies depending on the individual's age (Landi et al., 2003; Kovaleva et al., 2008). Besides the main ions of Ca^{2+}, PO_4^{3-}, OH^- and CO_3^{2-}, there are other of minority ions which includes Mg^{2+}, K^+, Na^+, F^-, and Cl^- as reported by LeGeros (1994) and Slosarczyk et al. (2010). This is depicted in Table 2.1 showing the elements of adult human hard tissues.

Table 2.1 Human hard tissue components of the human adult (LeGeros, 1994)

Constituent	Composition (wt%)		
	Bone	Enamel	Dentin
Calcium, Ca^{2+}	34.8	36.5	35.1
Phosphorus, P	15.2	17.7	16.9
Sodium, Na^{+}	0.9	0.5	0.6
Magnesium, Mg^{2+}	0.72	0.44	1.23
Potassium, K^{+}	0.03	0.08	0.05
Carbonate, CO_3^{2-}	7.4	3.5	5.6
Fluoride, F^{-}	0.03	0.01	0.06
Chloride, Cl^{-}	0.13	0.30	0.01
Pyrophosphate, $P_3O_7^{4-}$	0.07	0.022	0.1
Total inorganic	65	97	70
Total organic	25	1.5	20
Absorbed H_2O	10	1.5	10

2.2 Types of bones

In general, bones in human and other mammal bodies can be classified into two types that are the cortical bone and trabecular bone (Hench & Wilson, 1993). The cross section of the human bone is shown in Fig. 2.2 (Bandyopadyay et al., 2006). Porosity and the unit microstructure determine the two types of bones.

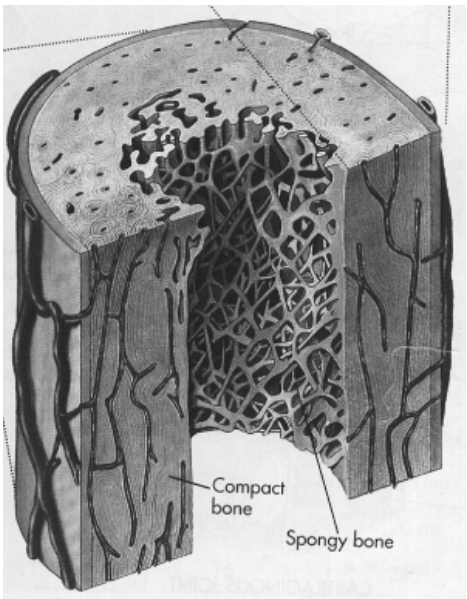

Fig. 2.2 Cross- sectional view of human bone (Bandyopadyay et al., 2006)

2.2.1 Cortical Bone

The cortical bone, also called the compact bone is the denser bone, consisting of parallel cylindrical units with porosity ranging between 5 to 10%. Cortical bone is primarily found in the shaft of long bones (femur, tibia, fibula, etc.) and forms the outer shell around the cancellous bone at the end of joints and the vertebrate (Meyes et al., 2008). The density of the cortical bone is reported to be around 1.99 g/cm^3 (Currey, 1998). Safadi et al. (2009) defined cortical bone as the dense hard, calcified bone that forms the hard outer "shell" of a bone which surrounds the marrow cavity.

2.2.2 Trabecular Bone

The trabecular bone or the cancellous bone, is the spongy type of bone. Its porosity ranges from 50 to 90%. It is normally found at the end of the long bones in vertebrate and in flat bones like the pelvis. Trabecular bone is like honeycomb in cross section and it is composed of short struts of bone material called trabeculae. The mechanical properties of trabecular bone are greatly dependent on its porosity and the way this porosity is structured. Besides that, the pores also perform other physiological functions and contain the marrow. Thus, bone is truly considered as multifunctional material (Hench & Wilson 1993; Meyes et al., 2008).

2.2.3 Cortical bone versus Trabecular bone

The major mechanical property difference between trabecular and cortical bone is the effective stiffness. Trabecular bone is more compliant than cortical bone and it is believed to distribute and dissipate the energy from articular contact loads. Only 20% of the skeletal mass within the body was contributed by trabecular bone while cortical bone contributes the remaining 80%. Besides that, the trabecular bone has much greater surface area than cortical bone. A comparison between the general

features and mechanical properties of cortical bone and trabecular bone is listed in Table 2.2. Generally, the compressive strength, flexural strength and Young Modulus of cortical bones are higher than that of trabecular bone.

Table 2.2 Comparison between structural features and mechanical properties of cortical bone and trabecular bone (Hench & 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

2.3 Bone Graft Substitute (BGS)

As discussed earlier, bone injury or damage can occur in many different ways, which include trauma, infections, tumours and metabolisms (Sutherland & Bostrom, 2003). It is known that skeletons support the body and also protection of vital organs, however, it is also susceptible to fractures as a result of injury and degenerative diseases which are often associated with aging. Thus, since the earliest of time, there has always been a need for the repair of damaged hard tissues (Best et al., 2008). Hence, bone graft substitute (BGS) has become critical in orthopaedic surgery.

Bone graft, firstly introduced in the 1800's with the earliest attempt was to replace missing bone with biomaterials, aimed to restore basic functions by repairing the defects due to injury or diseases (Czitrom & Gross, 1992; Meeder & Eggers,

1994). Bone grafts are generally used in combination with fixation devices in order to provide the mechanical stabilization during the healing process. Ideally, the graft should be able to replace the missing parts as well as to promote new bone in growth into the grafted area. This would reinforce the repaired area and at the same time building an interlock between natural bone and the grafted material. With time, the newly formed bone should also penetrate and replace much of the graft through a process called "remodelling" (Hing, 2005; Best et al., 2008).

Theoretically bone graft source can be adopted from another part of the patient's body (autograft), from another individual tissue obtained from a bone bank (allograft), from other mammalian species (xenograft) or from synthetic biomaterial (Czitrom & Gross, 1992; Meeder & Eggers, 1994, LeGeros et al., 2006).

2.3.1 Autogenous Bone Grafting

Autogenous bone grafting, also referred as autografting is regarded as the "golden standard" of bone grafting, as bone from another anatomic site of the patient is use to treat the defected bone of the patient himself (Togawa et al., 2004).

In autograft reconstruction, there will be no risk of transmission of diseases while the human body would accept its body's component better from a donor. The fact that there is no risk transmission is very positive for autograft and is widely accepted among the patients as reported by Hing (2005).

However, autograft has several serious drawbacks where, the volume of bone that can be harvested from the patient is limited and the collectable bone is also limited in its form. Mata et al. (2002) and Ishikawa et al. (2003) reported that this method usually requires secondary operation procedure and this would be costly, time consuming and sometimes it might cause additional further trauma. Besides

that, autograft could also cause donor site morbidity, patella fractures, patella tendonitis and scar formation (Burg et al., 2000, Ana et al., 2010).

2.3.2 Allogeneic Bone Grafting

Alternatively, allograft bone can be used where the bone graft source is adopted from donors. Modern allografting uses the material stored within the regulated bone banks (Sutherland & Bostrom, 2003). Generally, freeze-drying of the allograft is used to decrease their antigenicity as well as to have extended period of time (Whang & Wang, 2003).

Using allograft tendon will allow the patient to avoid donor site morbidity, reduce surgical time, smaller incisions and availability of graft either in terms of cancellous, cortical or combination of each bone. Elderly people (over 45 years of age), those requiring repairmen of the bones and skeletally immature athletes are common candidates for allograft tendons. Allograft reconstruction also provides a safer choice to those patients who are at higher risk of complication under anaesthesia (Sutherland & Bostrom, 2003; Hing, 2005).

Nevertheless, tissue availability, graft cost, delayed graft incorporation, sterilization, and long term graft strength are some issues related to allograft. Disease transmission is also a major concern in allograft tissue (Bostrom & Mikos, 1997; Burg et al., 2000). Two diseases which are most feared patients that would receive allograft tissue are human immunodeficiency virus (HIV) and hepatitis, although the risks of transmitting these diseases are relatively small. Most of the time, there is delayed graft incorporation because the body fails to accept the foreign tissue. Besides that, blood group-incompatible in bone transplantation can also cause the development of antibodies within ABO systems (Schnettler et al., 2004).

2.3.3 Xenotransplantation

The transplantation of living cells, tissues or organs from other mammalian species to human is known as Xenotransplantation. Such cells, tissues or organs are called xenografts or xenotransplants. Normally, bone tissue is taken from cows or pigs as sources for xenograft. Similar with allograft, xenograft has high potential of transmitting diseases and cause permanent alteration to the genetic code of animals Vagaska et al., 2010). Pigs also have a shorter lifespan than human thus, their tissues ages at a different rate (Tancred et al., 1998).

Xenograft also has been a controversial issue since they were first attempted. The animal groups strongly oppose killing animals in order to harvest their organ for human use. There are also religious beliefs (Muslim, Jewish) which prohibits the use of pigs as xenograft source (Boneva et al., 2001; Sopyan, 2009).

2.4 Biomaterials

Since there are serious drawbacks with bone graft substitutes, there has been requirement to produce synthetic materials. Hence, biomaterial is a synthetic material which is synthesized, used to replace part of living system and to function in intimate contact with living tissues (Park & Bronzino, 2003). It is a systematically and pharmacologically inert substance designed for implantation within or incorporation with living systems. Biomaterial is also defined as synthetic tissues intended to interface with the biological systems which would evaluate, treat, or replace any tissue, organ, or function of the body. By contrast, biological material is the bone matrix or tooth enamel, which is produced by a biological system.

There is always confusion between biomaterial and artificial material. The latter are those materials which are in contact with the skin, for example the hearing

aids and wearable artificial limbs. Artificial material is not biomaterial since the skin act as the barrier with the external world (William, 1992; Park & Lakes, 2007). On the other hand, biomaterials are used in the body system, typically in contact with inner tissues.

The basic functions of biomaterials are to assist in healing defects, to correct abnormalities and to improve function (Vagaska et al., 2010). Biomaterials also play important roles in order to repair and reconstruction of damaged and worn out part of the human body or that caused by disease. The function and example of biomaterials in human's organ are summarized in Table 2.3.

Table 2.3 Example and function of biomaterials in human's organ

(Park & Lakes, 2007)

Function	Example	Organ
Replacement of diseased or damaged part	Artificial hip joint, kidney dialysis machine	Bone, kidney
Assist in healing	Sutures, bone plates and screws	Bone
Improve function	Cardiac pacemaker, contact lens	Heart, Eye
Aid to diagnosis	Probes, catheters	Bladder

The successful implant of biomaterial in the body depends on factors such as the material properties, biocompatibility of the material used and design. The body's immune system rejection of the implant and the unwanted effect of the implant upon the body can cause failure of the biomaterial, which leads to toxicity, inducing an inflammation and causing cancer. In order to be classified as biomaterial, the materials have to meet the following requirements, (1) Non-toxic; (2) Non-carcinogenic; (3) Non-allergic; (4) Non-inflammatory; (5) Biocompatible; and (6) Bio-functional for life time (Desai et al., 2008). Table 2.4 list the classes and

example of materials used biomaterials, with their advantages and disadvantages, respectively.

Table 2.4 Class of materials used as biomaterials (Bhat, 2005; Park & Lakes, 2007)

Materials	Advantages	Disadvantages	Examples
Polymer (nylon, silicones, teflons)	• Light • Resilient • Easy to fabricate	• Deform with time • May degrade • Low mechanical strength	Sutures, blood vessels, other soft tissue, hip socket
Metals & Alloys (Titanium and its alloys, stainless steel, gold, cobalt-chromium)	• High impact strength • Tough • Ductile • High resistance to wear	• May corrode • Difficult to fabricate • Low biocompatibility	Joint replacement, bone plates and screw, dental root implants, orthopaedic load bearing
Ceramics (Alumina, zirconia, calcium phosphate)	• Good biocompatibility • Inert • Corrosion resistance • Biodegradable	• Brittle • Not resilient • Weak in tension • Special technique are needed for fabrication	Dental and orthopaedic implants, hip and knee prostheses
Composite (Carbon-carbon, wire-or fibre reinforced bone cement)	• Strong • Tailor made	• Difficult to make	Bone cement, dental resin

2.5 Bioceramics

Ceramic is defined as the art and science of making and using solid articles that have their essential components as inorganic and non-metallic materials that are been heat-treated (Kingery et al., 1976). In the past several decades, however, revolution had occurred in the use of ceramics to improve the health quality of human life. Researchers have developed a series of specially designed and fabricated ceramics for medical devices, and these are now referred to as bioceramics. Hence, the class of ceramics used for repair and replacement of diseased and damaged parts of human musculoskeletal systems are known as bioceramics (Thamaraiselvi & Rajeswari, 2004). This include the use as to repair and reconstruction of arthritic or fractured joints cause by damaged or diseased of the human body, correct chronic spinal curvature and immobilize vertebrae in order to protect spinal cord. It is also used to replace parts of cardiovascular system, especially heart valves, and therapeutically for the treatment of tumours. The biomedical application of bioceramics is summarized in Table 2.5.

Table 2.5 Biomedical Applications of Bioceramics
(Thamaraiselvi & Rajeswari, 2004)

Devices	Fuction	Biomaterial
Artificial total hip, knee, sheldow, elbow, wrist	Reconstruct arthritic or fractured joints	High-density alumina, metal bioglass coatings
Bone plates, screws, wires	Repair fractures	Bioglass-metal fiber composite
Permanently implanted artificial limbs	Replace missing extremities	Polysulfone-carbon fiber composite
Vertebrae spacers and extendors	Correct congesnital deformity	Alumina
Spinal fusion	Immobilize vetebrae to protect spinal cord	Bioglass
End osseous tooth replacement implants	Replaced disease, damaged or loosened teeth	Alumina, dense hydroxyapatite
Orthocontic anchors	Provide posts for stress application required to change deformities	Bioglass coated alumina

Bilotte (2003) reported that, bioceramics show better biocompatibility with tissue response when compared to polymeric or metallic biomaterials, and subsequently, they are being used as implants within bones, joints and teeth in the form of bulk materials of specific shape. However, despite their biocompatibility, there are also serious drawbacks where bioceramics are also known to be brittle, having low mechanical strength (in tension) and inferior workability. As a result, bioceramics are susceptible to failure with notches or microcracks because they do not deform plastically, and would fail easily (Bilotte, 2003).

2.5.1 Biocompatibility

The term biocompatibility relates to the ability of the material to elicit appropriate biological response in the given application. This include, as minimal as possible, any adverse reactions which may ensue at the blood/material or tissue/material must be with high resistance to biodegradation. The surrounding environment should not cause degradation or corrosion of the biomaterial as this could result in loss of physical and mechanical properties (Wise, 1995). The biocompatibility of the implant is influenced by several factors, such as implant shape, size, material composition, roughness, charge and surface wettability. Biocompatible is believed to be in a dynamic mode and ongoing process rather than static (Brantley & Eliades, 2001).

Besides that, biocompatibility of the biomaterial should also mean it would not cause thrombus-formation, cause adverse immune response and alter plasma proteins so as to trigger undesirable reaction (Park & Bronzino, 2003).

2.5.2 Classification of Bioceramics

As mention earlier, bioceramics have been established as a group of materials for medical application, mainly for implants in orthopaedic surgery, maxillofacial surgery and for dental implants (Thamaraiselvi & Rajeswari, 2004). This is due to their unique tissue responses, which in principle are three types, *i.e*. bioinert ceramics (nearly inactive with the environment), bioactive ceramics (form direct chemical bonds with the living organism), and bioresorbable ceramics (actively participate in the metabolic processes of an organism). The classification of implant-tissue interactions are summarized in Table 2.6 (Hench & Wilson, 1993). Generally, based on these types, there would be tissue response, either the tissue dies, form interfacial bonds or replace the implant (Hench & Paschall, 1973; Black, 1984).

Table 2.6 Classification of Implant-tissue responses (Hench & Wilson, 1993).

Classification	Tissue response	Implant/ tissue bond	Examples
Toxic	Tissue dies	None	Lead oxide, arsenic oxide
Biological nearly inert	Tissue forms an adherent fibrous capsule around the implant	None	Alumina, Zirconia and Carbon
Bioactive	Tissue forms an interfacial bond with implant	Chemical	Hydroxyapatite, Bio-glass, A-W glass
Bioresorbable	Tissue replace implant	Chemical	Carbonated hydroxyapatite, β-tricalcium phosphate, Calcium carbonate

Based on their chemical reactivity in a physiological environment as shown in Fig. 2.3, bioresorbable ceramics possess the higher relative reactivity followed by bioactive ceramics, while bioinert ceramics like alumina have essentially low relative reactivity (Shakelford, 1999).

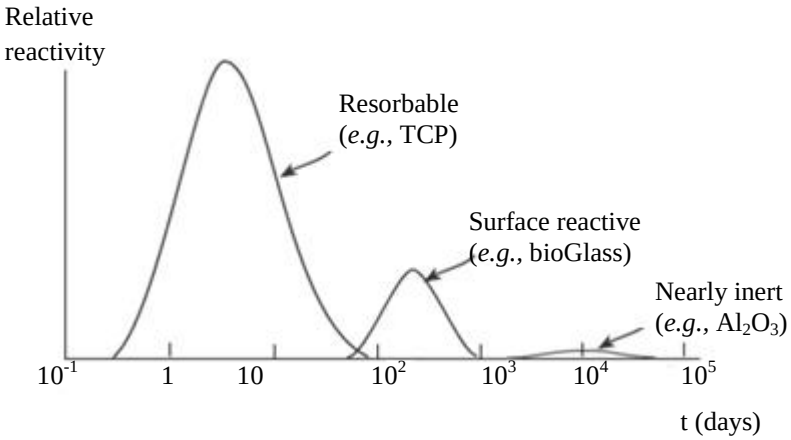

Fig. 2.3 Relative reactivity of different type bioceramics (Shakelford, 1999)

2.6 Calcium Phosphate Bioceramics

Chemical similarity to the mineral component of bones and teeth is the main reason for the use of calcium phosphates as bone graft substitute sources (Dorozhkin, 2009). Calcium phosphate is known to be non-toxic, biocompatible, it is not recognized as alien in the body and more importantly, by a "remodelling" process, the calcium orthophosphate can integrate into living tissue. This will lead to an intimate physicochemical bond between the implants and bone or named as osteointegration (Ong & Chan, 1999), hence resulting in a positive response of tissues leading to bone repair.

There are several types of calcium orthophosphate present and these are listed in Table 2.7. Different Ca:P molar ratio of each compound would result in different types of calcium phosphates such as di-, tri-, and tetra- calcium phosphate, hydroxyapatite, fluoroapatite, carbonated hydroxyapatite and β-whitlockite. The properties of these various calcium phosphates are different and hence they are used in different applications, as indicated in the same table. Bioresorption and bioactivity are the most important properties of calcium phosphate, and they are essentially dynamic and strongly depend on biological parameters. This includes the interaction

with collagen and accumulation of proteins and cells on the surface of the material, subsequently followed by resorption of the material and formation of bone (Thamaraiselvi & Rajeswari, 2004).

Table 2.7 Calcium phosphate used as biomaterials
(LeGeros & LeGeros, 2003)

Compound	Chemical formula	Ca: P molar ratio	Applications
Monocalcium phosphate monohydrate (MCPM)	$Ca(H_2PO_4)_2$	0.5	Cements, polyphosphates
Dicalcium phosphate dehydrate (DCPD)	$CaHPO_4.2H_2O$	1.0	Cement, coating
Dicalcium phosphate anhydrous (DCPA)	$CaHPO_4$	1.0	Cement, coating
Octacalcium phosphate (OCP)	$Ca_8H_2(PO_4)_6.5H_2O$	1.33	Coating, bonegraft
Alpha tricalcium phosphate (α- TCP)	$Ca_3(PO_4)_2$	1.50	Cements
Beta tricalcium phosphate (β- TCP)	$Ca_3(PO_4)_2$	1.50	Cement, bone graft composites
Calcium deficient apatite (CDA)	$Ca_3(PO_4)_2$	< 1.67	Cement, bone graft composites
Hydroxyapatite (HA)	$Ca_{10}(PO_4)_6(OH)_2$	1.67	Bone graft, coatings
Fluoroapatite (FA)	$Ca_{10}(PO_4)_6F_2$	1.67	Bone graft, coatings
Carbonated hydroxyapatite (CHA)	$(Ca, Na)_{10}(PO_4, CO_3)_6(OH)_2$	1.7- 2.6	Bonegraft
Tetracalcium phosphate (TTCP)	$Ca(PO_4)_2O$	2.0	Cements

The stability of calcium phosphate is reported to have increased with increasing Ca:P molar ratio (Bilotte, 2003). Slight differences in composition and structure of calcium phosphate compounds may have significant effect on their *in vivo* behaviour. Bohner (2000) reported that the main characteristic of calcium phosphate is probably its solubility in water because *in vivo* behaviour of calcium phosphate can be predicted to a large extent by their solubility. If calcium phosphate

has lower solubility than the mineral bone, the calcium phosphate will degrade extremely slow and vice versa.

The behaviour of calcium phosphate biomaterial in biological environment determines how they can be used *in vivo*. The most important requirement for calcium phosphate to be bioactive and bond to living tissue is the formation of bone like apatite layer on their surface. This phenomenon can be mimically *in vitro* by using chemically simulated body fluid (SBF), a protein-free solution with ion concentrations similar to those of human blood plasma (Tas, 2000; Thian et al., 2002; Kukobo & Takadama, 2006). Bioactive calcium phosphate would typically result in apatite formation on the synthetic bone. Besides that, calcium phosphate has also been reported to support osteoblast adhesion and proliferation (Davies, 1996; Anselme, 2000).

Nevertheless, the limitations to apply calcium phosphates as load-bearing biomaterials are their mechanical properties; that is, they are essentially brittle with a poor fatigue resistance (Suchanek & Yoshimura, 1998; Hench, 1998). For this reason, calcium phosphate is currently being used as fillers and coatings in biomedical application (Dorozhkin, 2009). However, there are wide variations in the mechanical properties of synthetic calcium phosphate, and these are attributed to the variations in the structure of polycrystalline calcium phosphate due to variations in manufacturing process (Bilotte, 2003).

Park and Lakes (2007) reported that the formations of calcium phosphate compounds are not only dependent on Ca:P molar ratio but also on the presence of water, impurities, and temperature. In wet environment and at lower temperature (<900 °C), it is more likely that the hydroxyapatite will form while in dry

atmosphere and higher temperature calcium phosphate will start to undergo phase transformation and form tri-calcium phosphate (α- and β-) , tetracalcium phosphate and calcium oxide. In many cases, both types of structures exist in the final product, or what is known as biphasic apatite. Table 2.8 shows the general physical properties of calcium phosphate.

Table 2.8 Physical properties of Calcium Phosphate (Park& Bronzino, 2003)

Properties	Value
Elastic Modulus (GPa)	4.0- 117
Compressive Strength (MPa)	294
Bending Strength (MPa)	147
Vickers Hardness (GPa)	3.43
Poisson's Ratio	0.27
Theoritical density (g/ cm^3)	3.16

2.6.1 Micron-Sized and Submicron-Sized Versus Nanodimensional Calcium Orthophosphates

Nanostructured materials are then defined as materials having structural elements with dimensions being less than 100 nm range (Moriarty, 2000). As such, nanocoatings would have individual layers or multilayer surface coatings in the range of 1-100 nm thick, nanofibers are fibers with diameter up to 100 nm and nanopowders are those with an average particle size of less than 100 nm. The advantages of nanodimensional calcium phosphate versus coarser crystals (micron- and submicron-sized) are shown in Fig. 2.4. These include solubility, densification, bioactivity, mechanical, and chemical properties (Stupp & Ciegler, 1992; Webster et al., 2001; Huang et al., 2004; Wang & Shaw, 2009).