DEVELOPMENT OF METAL DOPED HYDROXYAPATITE FOR BONE IMPLANT APPLICATION

SHARIFAH ADZILA BINTI SYED ABU BAKAR

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FACULTY OF ENGINEERING
UNIVERSITY OF MALAYA
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ABSTRACT

Hydroxyapatite (HA) powder with nanosize particles was successfully synthesized by mechanochemical method in a dry state at 170 rpm, 270 rpm and 370 rpm rotation speeds in 15 hours respectively. The research revealed that among the three rotation speeds employed, 370 rpm synthesized powder showed better characteristics as determined by X-ray Diffraction (XRD), Fourier Transform Infra Red (FTIR) and Field Emission Scanning Electron Microscopy (FESEM) analyses. Similarly, the sintering properties of these powders showed maximum densification, Vickers hardness and fracture toughness with values of 96.8%, 5.29 GPa and 1.49 MPa.m$^{1/2}$, respectively were obtained when sintered at 1250°C. The rotation speed of 370 rpm was subsequently employed to prepare the Na-doped HA and Mg-doped HA. The study found that the decrease of the XRD peak intensities and the decrease of FTIR adsorption bands corresponded to HA phase were related to the increase of Na$^+$ and Mg$^{2+}$ ions concentration. The substitution of the ions also changed the lattice parameters, the unit cell volumes and the crystal size of HA. Sintering and ion doping also influenced the stability of the HA phase where the decomposition occurred between 1000°C-1300°C sintering temperature and with increasing Na$^+$ and Mg$^{2+}$ ions concentration in the HA lattice. This has led to the increase of HA phase decomposition due to Na$^+$ and Mg$^{2+}$ replacing the Ca$^{2+}$ sites. Increasing sintering temperature has led to the decrease of relative densities, Vickers hardness and fracture toughness of undoped HA, Na-doped HA and Mg-doped HA. The 1% Na-doped HA was found to have maximum densification and Vickers hardness of 99.2% and 5.47 GPa, respectively when sintered at 1250°C. The 1% Na-doped HA also exhibited a high fracture toughness of 1.84 MPa.m$^{1/2}$ when sintered at 1300°C. Na$^+$ doping was more effective in enhancing the mechanical performance of HA compared to Mg$^{2+}$ doping. Na-doped HA and Mg-doped HA increased the biocompatibility of HA as the maximum growth of calcium phosphate was significantly shown in the 7% Na-doped HA after 7 days of exposure. However, osteoblast cell culture showed that increasing Na$^+$ and Mg$^{2+}$ concentration to 7% – 9% molar concentration induced the formation of dead cells near to the samples. The study found that Na$^+$ and Mg$^{2+}$ in higher molar concentration particularly 9% doping was detrimental as it affected the mechanical properties as well as the biocompatibility of the hydroxyapatite ceramic.
ABSTRAK

Serbuk hidroksiapatit dengan zarah saiz nano telah Berjaya dihasilkan melalui kaedah mekanokimia pada kelajuan putaran 170 rpm, 270 rpm dan 370 rpm dalam 15 jam. Kajian telah menunjukkan antara ketiga-tiga kelajuan putaran yang digunakan, serbuk yang disintesis pada 370 rpm telah menunjukkan pencirian yang lebih baik melalui analisis X-ray Diffraction (XRD), Fourier Transform Infra Red (FTIR) dan Field Emission Scanning Electron Microscope (FESEM). Sifat-sifat pensinteran serbuk yang disintesis pada 370 rpm ini juga menunjukkan pemadatan, Vickers kekerasan dan keliatan patah yang maksimum dengan 96.8%, 5.29 GPa dan 1.49 MPa.m$^{1/2}$, di perolehi masing-masing apabila disinter pada suhu 1250°C. Bagi logam dop, 370 rpm digunakan untuk sintesis Na-dop HA dan Mg-dop HA dalam 1% - 9mol% kandungan masing-masing. Na-dop HA dan Mg-dop HA telah berjaya disintesis melalui kaedah yang sama di mana penurunan keamatan puncak dan penurunan kumpulan penjerapan telah menunjukkan kesepadanan dengan fasa HA dengan peningkatan kandungan Na$^+$ dan Mg$^{2+}$ ion melalui analisis XRD dan FTIR. Penggantian ion juga telah mengubah parameter kekisi, isipadu unit sel dan saiz kristal HA. Pensinterandan ion-ion dop juga mempengaruhi kestabilan HA di mana penguraian berlaku antara suhu pensinteran 1000°C - 1300°C dan peningkatan kandungan ion-ion Na$^+$ dan Mg$^+$ telah membawa kepada peningkatan penguraian masing-masing kerana kekurangan kalsium berlaku disebabkan oleh penggantian ion-ion Na$^+$ dan Mg$^{2+}$ ke dalam kedudukan Ca$^{2+}$. Peningkatan suhu pensinteran telah membawa kepada penurunan ketumpatan relatif, Vickers kekerasan dan keliatan patah HA, Na-dop HA dan Mg-dop HA. 1% NaHA telah meningkatkan ciri-ciri HA di mana nilai maksimum pemadatan dan kekerasan Vickers, 99.2% dan 5.47 GPa telah diperolehi masing-masing pada 1250°C. 1% NaHA
juga telah meningkatkan keliatan patah kepada nilai maksimum, 1.84 MPa.m^{1/2} apabila disinter pada 1300\(^o\)C. Na\(^+\) ion nyata telah meningkat kanciri-ciri mekanikal HA berbanding Mg\(^{2+}\) ion dalam pelbagai kandungan. Na-dop HA dan Mg-dop HA meningkatkan kesesuaian bio HA di mana pertumbuhan maksimum kalsium fosfat telah ditunjukkan dalam 7% NaHA selepas 7 hari rendaman. Walau bagaimanapun, sel osteoblas t kultur telah menunjukkan bahawa peningkatan kandungan ion Na\(^+\) dan Mg\(^{2+}\) kepada 7 mol% - 9 mol% telah mendorong pembentukan sel-sel mati berhampiran dengan sampel. Secara keseluruhan, Na\(^+\) dan Mg\(^{2+}\) dalam kandungan mol yang tinggi terutamanya 9 mol% telah menunjukkan kelemahan prestasi mekanikal serta kesesuaian bioseramik hidroksiapatit.
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CHAPTER 1: INTRODUCTION AND OBJECTIVES

1.1 Biomaterials

The application of artificial implants started since 17th century with the Romans, using artificial legs made of wood to replace damaged legs/limbs of soldiers. In 1962, Charnley used low friction arthroplasty with Polytetra Fluoroethylene (PTFE) to replace damaged joints. Since then, much research was devoted in the development of artificial implants. Various types of materials including that of metallic, polymer and ceramic materials were used in artificial implants especially in the field of orthopedics (Nguyen and West, 2002). In 1980, the development of natural material implants have been introduced due to some problems with biocompatibility of artificial implants and the host cells. The naturally derived materials for biomedical application consists of autograft, allograft and xenograft (Nguyen & West, 2002; Yarlagadda et al., 2005).

Owing to the primary connection between living tissues from the same host the most successful bone grafting rates are attained using autografts. Any immune reaction is not found and the microscopic architecture is matched properly. Autograft has a sole drawback and that is it should be harvested from a secondary location of the patient’s body such as from the hip bone or tibia bone. This generally leads to greater misery and much complex surgery (Burg et al., 2000; Summers & Eisenstein, 1989; Tiedeman et al., 1995; Younger & Chapman, 1989). On the other hand, allograft is defined as grafting of tissues between similar species individuals under the condition that they must have dissimilar genetic composition while xenograft is defined as grafting of tissues between different species such as human and animal (Kumar, 2006; Mourino & Boccaccini, 2009). However these two types of bones have to undergo many different
treatment sequences for rendering it inactive to immune reactions and avoiding cross infection of the host diseases. Several forms of these treatments can be irradiation (Pekkarinen et al., 2005), freeze-drying (Heo et al., 2009) and acid washing (Peng et al., 2007).

The ability of biomaterials in the repair and the replacement of defect bones and joints have resulted in the development of special medical grade implant comprising of stainless steel, cobalt-chrome alloys, titanium-alloys, polyethylene (PE) and polymethyl-methacrylate (PMMA). However, such implants have been reported to fail before the patient dies (Yarlagadda et al., 2005). Thus, development of new biomaterials has been a great challenge in material science and engineering. Proper implants for hard tissue substitution should be bioactive (i.e. there is a chemical bond at the coalesce of the bone or implant), and must be tougher than the bone (Bonfield & Tanner, 1997). Apart from strength, the fitting implant material should biodegrade for a specific time period for allowing the replacement by the natural tissues (Rodeo et al., 2005; Tanner et al., 1994). This means that to withstand the load bearing applications, the ideal biomaterial should possess various suitable physical properties and ample strength. It should also be biocompatible to escape infection or rejection in body of the patient (Ni et al., 2007). To meet the growing demand for artificial bones and dentures the scientists have developed better biomaterials devices which are suitable for human biological environment and more significantly they do not impose detrimental reaction in the host tissues. Table 1.1 shows that biomaterials can be divided based on their reaction with surrounding tissues (Ni et al., 2007).

During implantation bioinert materials like titanium, alumina, zirconium, ultra-high molecular weight polyethylene (UHMWPE) and stainless steel might possess mechanical interlock with human bone. But they usually show minimum tissue response in the case where the bioinert implant interface is neither chemically nor biologically
bonded. There will be relative movement along with increasing growth of a non-adhering fibrous capsule of changing thickness (based on material and the scope of relative motion) in both soft and hard tissues (Sprio, 2008).

Table 1.1. Types and classification of biomaterial-tissue attachment.

<table>
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<tr>
<th>Implant categories</th>
<th>Attachment Classification</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almost inert</td>
<td>Mechanical interlock (morphological fixation)</td>
<td>Alumina, Zirconia, Titanium, Stainless Steel, UHMWPE</td>
</tr>
<tr>
<td>Porous</td>
<td>Ingrowth of tissues into pores (biological fixation)</td>
<td>Hydroxyapatite (HA), HA coated porous metals</td>
</tr>
<tr>
<td>Bioactive</td>
<td>Interfacial bonding with tissue (bioactive fixation)</td>
<td>Bioactive glasses, HA, Bioactive ceramics</td>
</tr>
<tr>
<td>Resorbable</td>
<td>Tissue replacement</td>
<td>TCP, Polylactic acid</td>
</tr>
</tbody>
</table>

(Ni et al., 2007)

This occurrence would eventually lead to deterioration in implantation and tissue function at the interface along with excessive movement at the surface of biomaterial tissue (Christel et al., 1988; Hulbert et al., 1987; Sprio, 2008). Loosening of bioinert implant invariably leads to clinical failure such as direct fracture of the implant or of the bone close to the implant (Hench, 1991). Bioinerts materials/implants that deformed or displaced after surgery could cause serious damage to the surrounding tissue (Bonfield & Tanner, 1997). During implantation into a human bony site, the biologically inactive biomaterials such as titanium (Ti) remains stable although titanium is considered as a foreign object in human body, it does not result an allergic reaction dissimilar to some stainless steel implants. Titanium possesses excellent corrosion resistance because it forms oxide layer on the surface. However, fabrication of titanium metal is quite crucial
as compared to stainless steel and it requires huge cost too. Ultra-high molecular weight polyethylene (UHMWPE) used as an implant materials from 1960's, is popular for using it as a surface for bearings in total joint prostheses. However, this material is often suffers from fatigue failure and it can produces many wear particles. These particles might result some inflammatory reactions and ultimately damage the healthy human cells (Figueiredo-Pina et al., 2009; Premnath et al., 1996; Renò et al., 2003).

Even porous implants exhibit biological fixation, the main limitation faced by this material is to have a pore diameter of about 100-150 μm for the tissues to remain healthy (Hulbert et al., 1987). If somehow micro-movement takes place at the porous implant interface, there is probability of tissues being damaged, interruption of blood supply and ensuing of inflammation. These may result in the loss of interfacial stability (Hench, 1998). On the other hand, the strength of material will decrease with increase in the proportion of large pores required for the development of bone growth (Liu, 1998; Ruys et al., 1995).

Bioactive materials help in the direct biochemical bonding to the neighboring tissues and also accelerate development of new bones, which have been widely used for repairing bone defects since it serves as a medium for load transfer and for living tissues and bones. Several bioactive materials are high density hydroxyapatite (HA), glass-ceramics A-W and certain bioglasses. Bioreabsorbable type of materials have also been used however, problems may evolve such as the retention of interface durability during the degradation period in order to allow replacement by natural host tissue (Hench, 1998). It is necessary that a resorbable biomaterial metabolically comprises only of substance which is sufficient for immune system of the body (Nilsson et al., 2004). A typical good example of a resorbable bioceramic is tricalcium phosphate and calcium sulphate hydroxyapatite (DeGroot. 1988; Nilsson et al., 2004; Zhang et al., 2001).
For the repair and replacement of diseased or injured body parts the unique use of specially designed ceramics has grown substantially since last two decades primarily because of its outstanding biocompatibility with bone tissue (Vallet-Regí & Gonzalez, 2004). These ceramics are termed as “bioceramics” (Hench, 1991).

1.2 Bioceramics

Ceramics consist of a large family of inorganic/non-metallic compositions. It is often used in orthopedic and dental applications (Ratner, 2001). Ceramics turned out to one of the most significant biomaterials used today because of its useful properties such as excellent strength and stiffness, remarkable corrosion and wear resistance, low density, etc. In fact, ceramics are biologically most common materials compared to others. Ceramics are usually entirely oxidized or chemically stable compounds (Nascimento et al., 2007). Because of their chemistry, ceramics have less probability of producing adverse effects, compared to metals and polymers, which possess low chemical resistance. Calcium phosphate ceramics (hydroxyapatite, tricalcium phosphate and tetracalcium phosphate), alumina, zirconia, silica based glasses (also called bioactive glasses). Pyrolytic carbons, these are used for replacing bone (Paul & Sharma, 2006). Alumina (Al₂O₃) which has wide application in orthopedic surgery cases since over 20 years has remarkable corrosion resistance, high strength, high wear resistance and good biocompatibility. Variation of these materials extends inert to bioactive depending on their biological activity. They can stay unaltered, or may dissolve or actively participate in physiological processes to improve formation of bone tissue. However, ceramics more specifically bioactive ceramics are brittle and unsuitable for load bearing applications for orthopedic implants (Pria, 2007).

Calcium phosphate materials are widely used for bone substitution and enlargement because of their resemblance with the mineral component of bone since
about past 30 years (Kalita & Ferguson, 2006; Mastrogiacomo et al., 2006; Ramanathan & Ackerman, 1999; Reid et al., 2006; Schneiders et al., 2007). Most calcium phosphates are considered as resorbable biomaterials. This is the reason of why they will dissolve under physiological conditions. The advantage of calcium phosphate biomaterials is that the dissolved products can be assimilated by the human body very rapidly. Apart from being non-toxic, these are biocompatible and more significantly, show bioactive behavior (Kalita & Ferguson, 2006; Vandiver et al., 2005; Weiss et al., 2005) being combined with the tissue by the same processes used in remodeling of healthy bone. This produces a close physicochemical bond between the calcium phosphate implants and bone, known as osseointegration (Albrektsson & Johansson, 2001).

Table 1.2 shows the various types of calcium phosphate which are classified according to their Ca/P ratio. In particular, hydroxyapatite (Ca$_{10}$(PO$_4$)$_6$(OH)$_2$, (HA) is the most stable calcium phosphate form (Chow, 2009), which has many biological benefits like bone bonding capability and accelerating new bone formation around the implant (Ishikawa et al., 1993). For stoichiometric HA the Ca/P ratio 1.67 assuming that no ionic type of substitution has occurred in the apatite structure (Fulmer et al., 1992).

<table>
<thead>
<tr>
<th>Ca:P</th>
<th>Mineral name</th>
<th>Formula</th>
<th>Chemical name</th>
</tr>
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<tbody>
<tr>
<td>1.00</td>
<td>Monenite</td>
<td>CaHPO$_4$</td>
<td>Dicalcium phosphate (DCP)</td>
</tr>
<tr>
<td>1.00</td>
<td>Brushite</td>
<td>CaHPO$_4$$\cdot$2H$_2$O</td>
<td>Dicalcium phosphate dihydrate (DCPD)</td>
</tr>
<tr>
<td>1.33</td>
<td>-</td>
<td>Ca$_8$(HPO$_4$)$_2$(PO$_4$)$_2$$\cdot$5H$_2$O</td>
<td>Octocalcium phosphate (OCP)</td>
</tr>
<tr>
<td>1.43</td>
<td>Whitlockite</td>
<td>Ca$_{10}$(HPO$_4$)$_2$(PO$_4$)$_6$</td>
<td>-</td>
</tr>
<tr>
<td>1.50</td>
<td>-</td>
<td>Ca$_3$(PO$_4$)$_2$</td>
<td>Tricalcium phosphate (TCP)</td>
</tr>
<tr>
<td>1.67</td>
<td>Hydroxyapatite</td>
<td>Ca$_{10}$(PO$_4$)$_6$(OH)$_2$</td>
<td>-</td>
</tr>
<tr>
<td>2.00</td>
<td>-</td>
<td>Ca$_4$P$_2$O$_9$</td>
<td>Tetracalcium phosphate</td>
</tr>
</tbody>
</table>

(Hench & Best, 2013; Phillips et al., 2000)
Because of chemical similarity with the hard tissue and the excellent biocompatibility and bioactivity properties (Cristea et al., 2003; Leventon, 2006; Mahabole et al., 2005; Prabakaran & Rajeswari, 2006; Vijayalakshmi et al., 2005), HA is extensively used in drug delivery systems and tissue engineering field (Fritsch et al., 2007; Loo et al., 2008; Oh et al., 2006). HA is also used as a bioactive ceramic coating in orthopedic and dental implants to contribute to their bone fixation (Darimont et al., 2002; Kannan et al., 2002; Oguchi et al., 1995) where this approach could enhance the integrity and durability of the implanted prosthesis (Gross et al., 2004). Clinically HA used as filler for gaps in bone and as implant in non-load bearing anatomic sites such as ocular implant and middle ear (Kundu et al., 2002). However, a major drawback of HA is its low fracture toughness (~ 1MPa m^{1/2}) (Tan, 2008) which renders this bioceramic to be used in non-load bearing application. Thus, this has posed a big challenge to scientist and researchers to improve the toughness of hydroxyapatite.

Current research focused on synthetic apatite including the replacement of chemical species obtained in natural bone. The fusion of the species is approximated to possess significant influence on the physical, chemical and physiological characteristics of the solid and therefore on the mineralization, demineralization and remineralization method of the cemented tissues. Lots of studies have focused on HA synthesis and biphasic calcium phosphate (BCP) with substituted Mn (Sopyan & Natasha, 2009), Zn (Webster et al., 2004), Sr (Kim et al., 2004) and Si (Porter et al., 2003). However, there were no studies focusing on the influence of these chemical elements on the mechanical properties of HA and also the effect of sintering process. Early studies have proved that the substitution of chemical species through various synthesis methods is very effective at diffusing these chemical elements into the BCP and reinforcement into HA. However, much work is now required in understanding and optimizing the process to
develop metal-doped HA and to investigate the effect of metal doping on the performance of dense hydroxyapatite.

Among the additives used, sodium (Na) and magnesium (Mg) usually have significant effect in biological apatites since they help in process of cell adhesion and bone metabolism and resorption process. Na\(^+\) (0.9 wt.\%) and Mg\(^{2+}\) (0.72 wt.\%) have been traced as an abundant element in natural bone and tooth mineral after calcium and phosphorous (Ginty et al., 1998). Studies suggest that Mg\(^{2+}\) accelerates osteoblast proliferation with an influence which can be compared to that of insulin (a known growth factor for osteoblast) (Riman et al., 2002). Methods like hydrolysis, double decomposition and solid state reaction exhibit that Na\(^+\) can be substituted in HA lattice at the calcium sites by producing supplementary vacancies (Aoki, 1991; De Maeyer et al., 1993; El Feki et al., 1999). Thus the precipitation method is mostly employed in synthesizing HA doped by Na\(^+\) and Mg\(^{2+}\) (Kannan et al., 2008; Laurencin et al., 2011; Ren et al., 2010; Webster et al., 2004).

Mechanochemical is a simple method to synthesize powders especially in dry condition whereby drying, filtering and calcination steps after synthesis are not necessary. Besides, less contamination is produced and thus making this method environmentally friendly. Hence, some researchers have employed mechanochemical synthesis in producing HA in wet medium (Mostafa, 2005; Rhee, 2002; Yeong et al., 2001) and in dry medium (González et al., 2006; Nasiri-Tabrizi, 2009; Silva et al., 2003). Since there are no studies conducted on preparing metal-doped HA under dry condition, the present study focused on dry mechanochemical synthesis of Na-doped HA and Mg-doped HA powders.
1.3 Objectives of the Research

1.3.1 To synthesis hydroxyapatite (HA), sodium (Na) – doped HA and magnesium (Mg) – doped HA powders through dry mechanochemical synthesis.

1.3.2 To characterize the effects of sodium (Na) doping and magnesium (Mg) doping on hydroxyapatite (HA) powder properties.

1.3.3 To investigate the effects of Na doping and Mg doping on the physical and mechanical properties of sintered hydroxyapatite.

1.3.4 To investigate the effects of sintering temperatures on the physical and mechanical properties of HA, Na – doped HA and Mg – doped HA.

1.3.5 To study the biocompatibility of HA, Na – doped HA and Mg – doped HA through in-vitro analysis.

1.4 Structure of the Thesis

Chapter 2 presents a review of metals doped hydroxyapatite (HA) where the introduction of metals is divided into three parts; monovalent, divalent and trivalent metals doped HA. The types of synthesis techniques such as precipitation, sol-gel and mechanochemical in preparing metal doped HA are discussed in this chapter. Moreover, the processing techniques of densification, sintering and bioactivity test of metal doped HA are also discussed.

The chemicals and equipments used in this study are described in Chapter 3. This chapter explains the synthesis technique of dry mechanochemical method with various rotation speed (170 rpm - 370 rpm). The selected optimum parameters from mechanochemical method are utilized to synthesis Na-doped HA and Mg-doped HA with various molar concentrations. The characterization techniques of the synthesized powders are explained through the use of several equipments (XRD, FTIR, TEM,
TGA). The densification of the synthesized powders is explained from the compaction process to the sintering process. Several tests and measurement techniques applied to the sintered compacts such as Vickers microhardness, relative density and fracture toughness are presented in this chapter. The in vitro tests consist of simulated body fluid (SBF) and osteoblast cell cultures are also described in this chapter.

The results and discussion are divided into two chapters: Chapter 4 and Chapter 5. Chapter 4 discussed the preliminary study of the dry mechanochemical synthesis of HA. The results of the characterization powders from various rotation speeds and milling times through phase analysis, functional group analysis, particle size and shape analysis and thermal stability are discussed and the optimum parameters from this method are determined.

In chapter 5, the comparison between HA, Na-doped HA and Mg-doped HA as well as the effect of Na$^+$ and Mg$^{2+}$ in HA are discussed in terms of their powder characteristics, physical behaviour, mechanical performance and biocompatibility. The biocompatibility work is discussed through the apatite formation and cell adhesion on sintered HA, Na-doped HA and Mg-doped HA as observed through scanning electron microscopy (SEM).

The conclusions of the research are presented in Chapter 6. The properties of the powders synthesized through dry mechanochemical method as well as the effects of Na$^+$ and Mg$^{2+}$ in HA properties in terms of physicochemical, mechanical, biocompatibility, and the sinterability of HA, Na-doped HA and Mg-doped HA are summarized. Some suggestions and further works are also presented in this chapter.

Finally in the appendices present the details of experimental equipment and the standard references of analysis as well as paper publications.
CHAPTER 2: LITERATURE REVIEW

2.1 Introduction to Hydroxyapatite

Bone is a natural organic–inorganic ceramic composite comprises of collagen filaments containing fixed, properly arrayed, nanocrystalline, rod-shaped inorganic materials usually of 25–50 nm length (Poinern et al., 2009; Zhou & Lee, 2011). Structural order in bone takes place at some stratified levels and shows the materials and mechanical properties of the components as shown in Figure 2.1. Hydroxyapatite (HA) is chemically similar to a very complex tissue with general formula Ca\(_{10}\)(OH)\(_2\)(PO\(_4\))\(_6\) which is an inorganic component of bone matrix. It is the elemental constituent of natural bone. In the case of bone formation, collagen controls HA mineralization. Collagen is a special protein consisting of an ionic group, and a dispersive group. The ionic group interacts with HA and the dispersive group, stabilizes HA in the physiological environment. However, dental structures consist of HA, which is needle-shaped and it is formed by using protein control. Approximately the total biological mineralization process is a crystallization process which is regulated by organic component (Zhou & Lee, 2011).
CHAPTER 2: LITERATURE REVIEW

2.1 Introduction to Hydroxyapatite

Bone is a natural organic–inorganic ceramic composite comprises of collagen filaments containing fixed, properly arrayed, nanocrystalline, rod-shaped inorganic materials usually of 25–50 nm length (Poinern et al., 2009; Zhou & Lee, 2011). Structural order in bone takes place at some stratified levels and shows the materials and mechanical properties of the components as shown in Figure 2.1. Hydroxyapatite (HA) is chemically similar to a very complex tissue with general formula Ca_{10}(OH)_{2}(PO_{4})_{6} which is an inorganic component of bone matrix. It is the elemental constituent of natural bone. In the case of bone formation, collagen controls HA mineralization. Collagen is a special protein consisting of an ionic group, and a dispersive group. The ionic group interacts with HA and the dispersive group, stabilizes HA in the physiological environment. However, dental structures consist of HA, which is needle-shaped and it is formed by using protein control. Approximately the total biological mineralization process is a crystallization process which is regulated by organic component (Zhou & Lee, 2011).
Figure 2.1. The hierarchical structure of bone at its different length scales. The microstructure of cortical bone comprises of osteons with Haversian canals and lamellae, and at the nanoscale, the structural units are collagen fibers consisting of bundles of mineralized collagen fibrils (Rogel et al., 2008; Zhou & Lee, 2011).

Synthetic HA do not show properties similar to the biological properties of bone. This is associated with the chemical composition of the bone, along with calcium and phosphate, hydrogenophosphate ions, carbonates ions, magnesium, sodium, and numerous other trace elements. These elements are regulating the entire performance of human bone (Mezahi et al., 2009). Hence, the resorption process of synthetic HA differs from that of natural bone essentially due to different textures (Bandyopadhyay et al., 2006). The space group of HA, Ca_{10}(PO_4)_6(OH)_2, was fixed as P63/m, and the unit cell of HA is displayed in Figure 2.2. Ten Ca^{2+} ions in the apatite were found to be located in two non-equivalent crystallographic sites: four at the Ca (1) aligned in the column, which is surrounded by nine oxygen atoms; six at the Ca (2) positions - one group of three calcium atoms forming a triangle which is spotted at 1/4 c, the other group of three at 3/4 c, surrounding the OH groups placed at the corners of the unit cell at 1/4 c and 3/4 c, respectively. Seven Ca (2) ions were found to be coordinated (with six O atoms from
$\text{PO}_4^{3-}$ and one O atom from OH$^-$. The local arrangement environments of Ca(1) and Ca(2) sites are exhibited in Figure 2.3 (a) and (b), respectively (Ren et al., 2010).

Figure 2.2. A unit cell of HA with atoms labelled according to the element and symmetric type (Ren et al., 2010).

Figure 2.3. Local coordination environments of (a) Ca(1) and (b) Ca(2) sites (Ren et al., 2010).

Extensive research has been conducted for using synthetic HA as a bone substitute and/or replacement in biomedical field because of the chemical similarity of HA to natural bone. Lately, HA has been used for different types of biomedical applications, along with matrices for drug release control and also used as bone tissue
engineering materials (Choi et al., 2013; Ginebra et al., 2006; Zhou & Lee, 2011). Synthetic HA shows strong affinity to host hard tissues. Chemical bonding with host tissue gives HA a better convenience in clinical function compared to most other bone substitutes such as metallic implants (Bauer et al., 1991; Sanosh et al., 2009; Zhou & Lee, 2011). Advantages of synthetic HA are biocompatibility, good osteoconductive and osteoinductive capabilities (Legeros, 1993; Poinern et al., 2009; Zhou & Lee, 2011). The literatures have shown that sintered HA exhibited great biocompatibility with elastic tissues like skin, muscle and gums. Because of these properties, HA is considered an ideal choice for orthopaedic and dental implants or components of implants where it has been extensively used to reconstruct hard tissues. Widely used applications are repairing of bone, augmentation of bone, coating on metallic implants or as fillers in bone or teeth (Böhner, 2000,2010; Dorozhkin, 2009; Ginebra et al., 2010; Johnson & Herschler, 2011; Zhou & Lee, 2011).

Hydroxyapatite-coated implants coordinate with the bone healing process quite well. This characteristic is called “osteophilic” nature (Nuss & Rechenberg, 2008; Thomas et al., 1987). This property results a good substrate for osteoblasts. The application of hydroxyapatite as a coating of implants, is usually frequent. However, it has been observed that it may cause osteolysis (bone dissolution) while exposing it to bone marrow and soft tissues (Bloebaum & Dupont, 1993; Nuss & Rechenberg, 2008). The hydroxyapatite wear debris is considered as the prime reason for failure of implant as its phagocytosis accelerates discharge of cytokines. Finally these products were considered to be responsible for (granulomatous) inflammation, disturbance in bone remodelling and local osteolysis (Nuss & Rechenberg, 2008). Because of the low mechanical strength of HA ceramics, it is not suitable for use in low load-bearing applications. Recent progress in nanotechnology have however focuses more on the
formation of nanosized HA and examining its properties on the nanoscale (Zhou & Lee, 2011).

Nanocrystalline HA powders show better sinterability and improved densification because of larger surface area, which may upgrade the fracture toughness, and additionally other mechanical properties (Legeros, 1993; Zhou & Lee, 2011). Furthermore, nano-HA, usually have better bioactivity as compared to coarser crystals (Stupp & Ciegler, 1992; Zhou & Lee, 2011). Thus, nano-HA particles can be used for engineered tissue implants with better biocompatibility instead of other implants. Nanotechnology might significantly aid in the progress of HA biomedical materials. HA nano- and micro-crystals with multiform morphologies (separated nanowires, nanorods, microspheres, microflowers and microsheets) have been successfully manufactured by using a number of powder processing techniques, including sol–gel synthesis (Bose & Saha, 2003; Kivrik & Ta, 1998; Shih et al., 2004; Zhou & Lee, 2011), solid state reactions (Young & Holcomb, 1982), co-precipitation (Bernard et al., 1999), hydrothermal reactions (Liu et al., 1997), microemulsion synthesis (Lim et al., 1997) and mechanochemical synthesis (Suchanek et al., 2002). With the rise demand to establish clean, non-toxic and environmentally friendly techniques, HA are often extracted using bioproducts such as corals, cuttlefish shells, natural gypsum, natural calcite and bovine bone (Murugan & Rao, 2002; Rocha et al., 2005; Zhou & Lee, 2011). Chemical analysis exhibits that these products, which are typically considered as biowaste, are good sources of calcium in the form of carbonates and oxide (Zhou & Lee, 2011).

Dense sintered form of calcium hydroxyapatite is clinically very significant for bone repair, tooth root replacement, augmentation of alveolar ridges, pulp capping, and maxillofacial reconstruction (Barralet et al., 2003; Legeros, 1993; Tang et al., 2009). If the starting powder is stoichiometric with better powder properties such as crystallinity,
agglomeration, and morphology then dense HA ceramics with better mechanical properties can be developed (Tang et al., 2009; Thangamani et al., 2002). Often, HA ceramics show insufficient mechanical properties because of their poor sinterability, especially in wet environments in terms of physiological conditions. Major progresses have been achieved in developing bioceramic microstructures, for getting implant material for hard tissue replacement with better mechanical properties during the past decade. Grain sizes may reduce from microscale to nanoscale in dense sintered materials. This is one of the main method to improve the mechanical and biological properties of HA-based bioceramic materials (Banerjee et al., 2007; Kingery et al., 1976; Tang et al., 2009). High sintering temperatures and long sintering time required for consolidation of HA powders often result in extreme coarsening of grain and decomposition of HA. These effects result in degradation of its mechanical properties (Gu et al., 2004). Nanostructured ceramics are usually processed by compressing nanopowders at high pressures and sintering them at different times and temperatures and in various atmospheres (Veljovic et al., 2007). Methods involving pressure, such as hot pressing, hot isostatic pressing, sinter forging, etc, are also applied to get nanostructured ceramic materials. Hot pressing helps to enhance densification kinetics and limit grain growth (Groza, 1999; Mayo, 1997; Raynaud et al., 2002a; Veljović et al., 2009). A high amount of β-TCP is very harmful for the sintering and mechanical properties of HA bioceramics (Raynaud et al., 2002a). To avoid degradation of the mechanical properties, β-TCP should not be present in HA bioceramic materials. Conversely, the rate of degradation and bioactivity for calcium phosphate ceramics can be regulated by combining HA and β-TCP (Tang et al., 2009).

To make strong bond between HA implant and surrounding bones, bone-like apatite layer can be formed on the implant surface by dissolution and re-precipitation process. This means that surface dissolution of HA is required to form an apatite layer
to some extent. Despite HA is the most stable ceramic material of calcium phosphates in biological medium, numerous studies have shown considerable dissolution of HA both in vitro and in vivo. A critical disadvantage of the severe dissolution is that mechanical degradation of HA itself adjacent to bone and decrease of the HA-bone interfacial strength reduce the probability of long-term mechanical stability of HA implant. Thus, dissolution properties of HA is one of the major issues in biomedical applications (Seo & Lee, 2008).

Besides the application as a bone implant, HA can also be used for industrial, technological and biotechnological purposes. It can be used as a catalyst in different reactions, like dehydration and decomposition of alcohols, methane oxidation and conversion of benzene to phenol. Besides, it is also used as adsorbents for separation of proteins and as an ion exchanger for many metal cations. In these cases, both bulk and surface structures and properties play significant roles (Mostafa & Brown, 2007). Recent years, chromatographic column filled with HA materials has been used in the purification and segregation of protein, peptide and antibodies, HA also has been used to adsorb heavy metal ions for the treatment of waste water (Matsumoto et al., 2004; Nie & Wang, 2007; Schubert & Freitag, 2007; Wang et al., 2010; Xu et al., 2007). In addition, HA was investigated aiming at a promising vehicle for drug delivery, like implantable materials, scaffolds of growth factors and antibiotics (Matsumoto et al., 2004; Nie & Wang, 2007; Saleem et al., 2005; Wang et al., 2010). HA microspheres (HA-MS) with high specific surface area can be formulated by spray-drying of aqueous suspension of nano-sized HA, which showed potential as the candidate carrier for sustained release of various active substances via subcutaneously or local tissue injection. It is well known that the adsorption/desorption (release) behaviours of drug molecules on HA is complicate and normally depend on the equilibrium concentration of the drug, pH, and other factors of the surrounded environment (e.g. the injection site).
Obvious initial burst release and only short-term release were observed when HA or HA-MS (HA-microsphere) was used as the vehicle for loading drugs compared to PLGA coated HA-MS which is shown in Figure 2.4 (a). The microstructure of HA-MS is shown in Figure 2.4 (b). Thus, the applications of HA in the continued drug delivery are limited, more specifically for small molecular, water-soluble drugs (Wang et al., 2010).

![Graph showing drug release over time](image)

**Figure 2.4.** (a) The in vitro release of Doxycycline Hydrochloride (Dox-HCl) from HA-MS and PLGA coated HA-MS over a period of 7 days. (b) HA-MS. The initial concentrations of Dox.HCl solution for adsorption were 2 mg/ml (Wang et al., 2010).

### 2.2 Metal Doped Hydroxyapatite

Although Hydroxyapatite (HA) has a good osseointegration and osteoconductive properties, the low mechanical properties such as low strength and brittleness (Fu & Chen, 2005) have limited its use in load-bearing application. Other drawbacks such as design limitations (Simon et al., 2005) and high level of crystallinity could result the nondegradability of pure HA when implanted in an organ (Xiu et al., 2005). Without accelerating the degradation process, the slow degradation of HA makes in vivo experiments in physiological conditions improbable. Bone tissue engineering is a specific section of nanotechnology where the nanostructured biomaterials may replace...
hard and soft skeletal tissue, and biocompatible materials for tissue genesis. Before creating a device which has the ability to mimic human bone, different bone types and their mechanical properties (Table 2.1) like compressive strength, Young’s modulus and fracture toughness etc. should be considered.

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<th>Table 2.1. Mechanical properties of skeletal tissues.</th>
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<td>Property</td>
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<td>Compressive strength (MPa)</td>
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<td>Flexural strength (MPa)</td>
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<td>Strain to failure (%)</td>
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<td>Fracture toughness (MPam^{1/2})</td>
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<td>Young’s modulus (GPa)</td>
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(Barinov, 2010)

Numerous researchers have tried to improve the mechanical properties of HA. One of the method is to dope HA with metal ions such as magnesium (Fadeev et al., 2003; Landi et al., 2008; Landi et al., 2006), manganese (Mayer et al., 2006), zinc (Laquerriere et al., 2006; Li et al., 2008), and strontium (Bigi et al., 2007; Fu & Chen, 2005; Kim et al., 2005; Verberckmoes et al., 2004). Those metals might affect the lattice parameters, the crystallinity, the dissolution kinetics and other physical properties of apatite (Li et al., 2008). Moreover, such metal ions play a significant role towards enhancing cell-material interactions of calcium phosphates and also upgrading its mechanical properties. Process of cell interaction on materials is very dynamic, and it encourages an exceptional tissue response at the biomaterial surface. Besides, minerals and traces of metal elements could help growth, and stimulate bone formation and resorption on bone cells or bone mineral in vivo (Saint-Jean et al., 2005) and in vitro (Burchfield et al., 2006). It has been also found that certain metal ions can penetrate into bacteria and deactivate their enzymes and could develop hydrogen peroxide which can kill bacteria or fungi (Kim et al., 1998). By utilizing the bioactivity of calcium
phosphates along with their superior mechanical properties of biocompatible metals, stronger and more stable biomaterials can be produced.

2.2.1 Monovalent Metals Doped Hydroxyapatite

Lithium (Li), sodium (Na) and potassium (K) are monovalent metal cations which are located in group 1A of the periodic table. Usually Li⁺ ions have their use as antidepressant while Na⁺ and K⁺ ions are present naturally in biological apatite in amounts of 0.9 wt. % and 0.03 wt. %, respectively (Barinov, 2010; Boanini et al., 2010). The substituted monovalent ions like Na⁺ and K⁺ for Ca²⁺ in the apatite structure have resulted the charge imbalance which can be neutralized by creating supplementary vacancies or by the simultaneous substitutions of cations and anions. Hence, it is clear that the hexagonal structure of HA can accommodates a wide variety of ions in its structure (Kannan et al., 2007b). Silver (Ag) is a transition metal ion often exists as monovalent ion and also shows strong inhibitory activities and strong antimicrobial effects on various bacteria. Additionally, this metal ion is superior to organic antimicrobial agents in terms of heat resistance, persistence of antimicrobial effects and safety (Matsumoto et al., 2009).

According to Wiesmann et al. (1998) potassium (K) significantly influenced the biomineralization process and several studies also have shown that K⁺ contributes in the continuation of biochemical process and serves in the apatite mineral nucleation process. Sodium (Na) has an important effect in biological apatites since it plays a potential role in a cell adhesion as well as in the bone metabolism and resorption process. Na⁺ has been detected as an abundant element in natural bone and tooth mineral after calcium and phosphorous (Ginty et al., 1998).

Generally, prosthesis implantation is subjected to bacterial infection mainly from Staphylococcus aureus (23%) and Coagulase-negative staphylococci (25%). This
problem have been reported in 1033 cases of total hip and total knee prosthetic arthroplasty infections (Steckelberg & Osmon, 1994). Silver (Ag) is considered more in research studies than other antimicrobial agents (copper, zinc, ampicilin and doxycycline) due to a wide-spectrum of antimicrobial properties (Feng et al., 2000). Although Ag⁺ can have an antibacterial effect in small percentage, it can also be toxic if large amount is present. It has been reported that the presence of 2 wt. % Ag⁺ in HA coating can have noteworthy antibacterial effect. Since then, one of the methods has been developed by Bai et al. (2010) where Ag⁺ was doped into HA by ion beam-assisted deposition (IBAD) method with the in situ heat treatment. Through this method, Ag⁺ was replaced into HA through the whole thickness to achieve the continuous release of Ag⁺ without osteoblast-precursor cell cytotoxicity. The silver particles sizes of 10-50 nm were spread throughout the coating thickness with bigger particles in the crystalline layer and smaller particles in the amorphous layer (Bai et al., 2010).

2.2.2 Divalent Metals Doped Hydroxyapatite

Calcium (Ca), magnesium (Mg), strontium (Sr), manganese (Mn), zinc (Zn), cobalt (Co) and silicon (Si) are divalent metals cations which are naturally present in human bone. Among of all these ions, Ca²⁺ is present in a great amount (36.51 wt %) (Barinov, 2010; Boanini et al., 2010). However, other divalent ions such as lead (Pb), nickel (Ni) and copper (Cu) are not present in human bone. Ca²⁺ is an important mineral for bones development. Mg²⁺ and Zn²⁺ usually reflect dietary history, but some ions can lead to an exposure to environmental hazards, like Pb²⁺ and Sr²⁺ where their substitutions can change the properties of HA (physico-chemical and mechanical) either causing or inhibiting normal function. Moreover, Zn²⁺ is found as the most effective dopant among all of divalent ions in enhancing osteoblast response to HA in terms of calcium deposition (Webster et al., 2004).
Strontium (Sr) ions possess many beneficial effects on bone formation (Saint-Jean et al., 2005) and prevention of bone resorption (Capuccini et al., 2008) through direct or indirect consequences on bone cells or bone mineral. Accordingly, Sr$^{2+}$ has been proved to possess a potential in a treatment of osteoporosis (Kim et al., 2004). In clinical studies, Sr$^{2+}$ imposed several effects on bone cells. In addition to its anti-resorptive activity, Sr$^{2+}$ was found to have anabolic activity in bone. This might lead to a better osteointegration when it is present in the biomaterials (Saint-Jean et al., 2005). Capuccini et al. (2008) used Sr-substituted HA as coating on titanium implant which is expected to enhance the bioactivity of the surface and stimulate bone opposition. Different concentrations of Sr (0, 5 and 10%) in HA thin films were stored on Ti substrates by Pulsed Laser Deposition (PLD). This method has quite a precise control over HA growth parameters at low deposition temperatures (Capuccini et al., 2008). Another preparation of Sr-HA coating titanium alloy substrate (Ti-6Al-4V) has been reported by Xue et al. (2007) via plasma spraying technique.

Zinc (Zn) is one of the trace metals present in the human apatite. Zinc is necessary for the development and growth of all species, including humans. It has been implicated with mineralization in biological systems (Lusvardi et al., 2002). Human bone contains about 0.0126 - 0.0217 wt% of zinc and it works as a cofactor for many enzymes and also essential for DNA replication (Li et al., 2008). The slow release of Zn$^{2+}$ added to an implant material could lead to the bone formation around the implant and enhances the bioactivity of HA (Lusvardi et al., 2002).

Manganese (Mn) is also one of the trace elements which has an influence on the stability and HA particle growth (Mayer et al., 2006). The addition of Mn$^{2+}$ ion into HA usually to promotes cell adhesion due to the activation of integrins by divalent Mn$^{2+}$ with extracellular matrix and cell surface ligands (Sopyan & Natasha, 2009). Hence, doping Mn$^{2+}$ into calcium phosphate phase is expected to exhibit improved mechanical
and biological performance in terms of the bioactivity of calcium phosphates (HA and BCP) and accelerate bone mineralization (Mayer et al., 2006).

Silicon (Si) exists less than 1 wt% in human body. It was found to be essential for normal bone cartilage growth and development as well as calcification (Gomes et al., 2010; Pietak et al., 2007). In mammalian systems, the existence of Si$^{2+}$ is variable according to the different organs such as 1 ppm in the serum; 2-10 ppm in the liver, kidney, lung and muscle; 100 ppm in the bone and ligaments; and 200-600 ppm in cartilage and other connective tissues. The high percentage of Si$^{2+}$ present in extracellular matrix components indicated a role for Si$^{2+}$ as a biological cross-linking agent. This contributes to the architecture and resilience of connective tissue. Si$^{2+}$ also has been used as a dietary supplement intake where the bone mineral density (BMD) is usually positively influenced by this element in men and premenopausal women. Earlier study reported that synthetic calcium phosphate (CaP) that have trace levels of Si$^{2+}$ in their structure significantly increased biological performance as compared to stoichiometric HA because of the direct effects of Si$^{2+}$ in physiological processes of the bone and connective tissue systems (Jugdaohsingh et al., 2004; Pietak et al., 2007). Currently two different Si-substituted CaPs have been commercially used in bone substitute applications; they are Actifuse TM (single phase Si-HA) and Skelite TM (multiphase Si stabilized CaPs mainly contained Si-$\alpha$-TCP), both are manufactured in micro porous scaffold and / or granule formats intended as fillers for bone defects in non-load bearing applications (Pietak et al., 2007).

Small amount of copper (Cu) was found to be essential in metabolic processes in most living organism (Stanic et al., 2010). It has a notable catalytic function in the first step of maturation of collagen to form stable fibrils. Cu$^{2+}$ was said to improve proliferation of endothelial cells and angiogenesis in-vivo which is significant in healing a large bone defects. Li et al. (2010) found that Cu$^{2+}$ ions tend to substitute the Ca(II)
sites since it has smaller radii (0.77 Å) than Ca (0.99 Å). Cobalt (Co) is known as a transition metal ions. Small quantity of Co is essentially indispensable in a living organism. But large amount of this element could cause the toxicity and debilitates the body immune system. Hence, too much metal ions can deteriorate cellular compartment of bone matrix and organic phase (Brodner et al., 1997).

Nickel (Ni) and lead (Pb) were found to influence the catalytic function of HA. Among the divalent metals, lead (Pb) is considered important in medical and environmental sciences. The problem with Pb$^{2+}$ is its poisoning effect in human. This has led to the curiosity of Pb$^{2+}$ uptake and retention in bone. Earlier study reported that Pb-doped HA is effective in oxidative coupling of methane to ethane at relatively low temperature of 700°C. Pb$^{2+}$ ions prefer to bound on the surface of HA which rapidly exchanged with Ca$^{2+}$ in solution to form Pb$_{10}$Ca$_6$(PO$_4$)$_6$(OH)$_2$ and followed by the formation of very stable and structurally different pyromorphite phase, Pb$_{10}$(PO$_4$)$_6$(OH)$_2$. Pb$^{2+}$ ions also has the tendency to substitute Ca (II) site and its anisotropic nature induced local lattice expansion (Ellis et al., 2006).

The involvement of magnesium (Mg) ions into HA structure is of great interest for the growth of artificial bone substitutes. Mg$^{2+}$ is one of the most important ions combined with biological calcium phosphates and plays significant role during spontaneous formation of in vivo calcium phosphate and bone bonding. Mg$^{2+}$ is closely associated with mineralization of calcified tissues, and this ion indirectly influences mineral metabolism (Adzila et al., 2012; Riman et al., 2002). The Mg$^{2+}$ shortage has an adverse effect on all stages of the skeletal metabolism, as it causes the discontinuation of bone growth, the reduction of the activity of osteoblasts and osteoclasts, osteopaenia and bone fragility. Chemical synthesis studies of partially Mg-substituted apatite show that in solution, Mg$^{2+}$ ion impeded the crystallization of the apatite, which resulted a synthetic apatite with a low level of crystallinity. This makes it even morphologically
REFERENCES

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