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DEVELOPMENT OF Co-Sr CARBONATED HYDROXYAPATITE FOR BIOMEDICAL APPLICATIONS

By

MUHAMMAD SYAZWAN BIN MOHD NOOR
Supervisor: Dr Yanny Marliana Binti Baba Ismail
Co-supervisor: Prof Dr Ahmad Fauzi Bin Mohd Noor

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DECLARATION

I hereby declare that I have conducted, completed the research work and written the dissertation entitles "**Development of Co-Sr Carbonated Hydroxyapatite for Biomedical Applications**". I also declare that it has not been previously submitted for the award of any degree or diploma or other similar title of this for any other examining body or university.

Name of Student: Muhammad Syazwan Bin Mohd Noor Signature:

Date : 3^{rd} July 2017

Witness by

Supervisor : Dr Yanny Marliana Binti Baba Ismail Signature:

Date : 3^{rd} July 2017

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LIST OF ABBREVIATIONS

BGS	Bone-Graft Substitute
CHA	Carbonated Hydroxyapatite
CHN	Carbon, Hydrogen and Nitrogen
Co	Cobalt
CO_2	Carbon dioxide
DTS	Diametral Tensile Strength
FESEM	Field Emission Scanning Electron Microscope
FTIR	Fourier Transform Infrared Spectroscopy
FWHM	Full Width at Half Maximum
HA	Hydroxyapatite
ICSD	International Centre of Standard Data
SBF	Simulated Body Fluid
Sr	Strontium
XRD	X-ray Diffraction
XRF	X-ray Fluorescence

LIST OF SYMBOLS

$^\circ$	Degree
θ	Theta
% D_s	Percentage of shrinkage in diameter
% t_s	Percentage of shrinkage in thickness
σ_{ult}	Ultimate tensile strength
%T	Percentage of Transmittance

PEMBANGUNAN Co-Sr HIDROKSIAPATIT TERKARBONAT UNTUK APLIKASI BIOPERUBATAN

ABSTRAK

Kobalt-strontium hidroksiapatit terkabonat (Co-Sr CHA) telah disintesis melalui kaedah pengemulsian nano. Ion Kobalt (Co) dan Strontium (Sr) diperkenal ke dalam struktur apatit untuk meningkatkan sifat-sifat mekanikal dan aktiviti biologi supaya hampir sama dengan mineral tulang. Serbuk CHA dijadikan rujukan untuk serbuk Co-Sr CHA sebagai perbandingan dalam kajian ini. Suhu sintesis yang sesuai untuk menghasilkan serbuk bersaiz nano dalam kajian ini adalah pada suhu bilik (RT). Empat komposisi berbeza serbuk Co-Sr CHA telah disintesis menggunakan teknik yang sama. Antara serbuk yang telah disintesis, Co-Sr CHA 1 dan Co-Sr CHA 2 telah dipilih sebagai komposisi optimum dan digunakan untuk fabrikasi produk tumpat. Pensinteran kemudian dilakukan ke atas sampel tumpat Co-Sr CHA pada suhu 900˚C dan disejuk dengan gas CO_2 kering pada suhu 200˚C. Semua sampel Co-Sr CHA tersinter didapati masih kekal sebagai jenis B-CHA. Penggunaan gas CO_2 kering didapati boleh mengantikan karbonat yang terurai semasa pensinteran pada suhu tinggi. Nilai ketumpatan relatif dan kekuatan tegangan lintang (DTS) yang diperolehi untuk Co-Sr CHA 1 masing-masing 89.15% dan 7.67 MPa dan ia menunjukkan bahawa Co-Sr CHA 1 mempunyai ciri-ciri mekanikal yang lebih baik jika dibandingkan dengan sampel CHA dan Co-Sr CHA 2 tersinter. Untuk ujian bioktiviti, terdapat pembentukan lapisan apatit pada permukaan sampel Co-Sr CHA selepas direndam dalam larutan SBF selama 7 hari. Co-Sr CHA 1 menunjukkan bioaktiviti yang baik dengan pembentukan cepat lapisan apatit jika dibandingkan dengan sampel tersinter lain. Jumlah kobalt dan strontium dalam struktur apatit mempengaruhi sifat mekanikal dan bioaktiviti produk. Oleh itu, penggantian kobalt dan strontium ke dalam struktur telah berjaya meningkatkan ciri-ciri bahan berasas CHA.

DEVELOPMENT OF Co-Sr CARBONATED HYDROXYAPATITE FOR BIOMEDICAL APPLICATIONS

ABSTRACT

Cobalt-strontium Carbonated Hydroxyapatite (Co-Sr CHA) was synthesized via nanoemulsion method. Cobalt (Co) and Strontium (Sr) ions were introduced into the apatite structure in order to improve their mechanical properties and enhance the biological activity which closely mimics to the bone mineral. CHA powders were used as a reference for Co-Sr CHA powders as a comparison in this study. The suitable synthesis temperature to produce nano-size powders in this study was at room temperature (RT). Four different compositions of Co-Sr CHA powders were synthesized using same technique. Among the as-synthesized powders, Co-Sr CHA 1 and Co-Sr CHA 2 were chosen as optimum composition and used for fabrication of dense products. Sintering was then performed on the dense samples of Co-Sr CHA at sintering temperature 900˚C and it cooled down with a dry CO_2 atmosphere at temperature 200˚C. All the sintered samples of Co-Sr CHA were found to retain as B-type CHA. The use of dry CO_2 atmosphere was found can compensate the carbonate loss during sintering at high temperature. The relative density and diametral tensile strength (DTS) values obtained for Co-Sr CHA 1 were 89.15% and 7.67 MPa respectively and it showed that Co-Sr CHA 1 has better mechanical properties if compared to sintered samples of CHA and Co-Sr CHA 2. For bioactivity test, the formation of apatite layer occurred on the surface of sintered samples of multi-doped CHA after soaking in SBF solution for 7 days. Co-Sr CHA 1 showed good bioactivity by the fastest formation of apatite layer if compared to other sintered samples. The amount of cobalt and strontium in the apatite structure were influenced the mechanical and bioactivity of the products. Thus, substitutions of cobalt and strontium in the structure successfully enhanced the properties of CHA-based material.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Bone applies important functions in the human body such as assist movement activity, ensure skeletons have sufficient bearing capacity and protection of internal organs (Florencio-Silva et al. 2015). Nowadays, accidents, injuries or diseases are a common phenomenon that makes bone susceptible to fracture. Fracture existences in bone are related to the quality of bone which influenced by several biological factors, the mechanical behavior and the microstructure (Kataruka et al. 2017).

Worldwide, the incidence of bone fractures is increasing dramatically and it influenced highly demand bone grafting ascended as the population age increases. Base from data collected from hospitals treating hip fractures, more than 300,000 hip and knee replacement surgeries have been performed by mostly senior citizens of age above 50. It has been estimated that by the year 2050, the frequency of bone fractures will reach 3.25 million in Asia due to an increase in the elderly population. In the United States, approximately 1.5 million fractures are attributed to osteoporosis. In Malaysia, the statistics have shown that the overall incidence of hip fractures about 90 per 100 000 individuals. Race-specific incidence data showed that the fracture rates and the highest patients come from the Chinese (160 per 100 000) followed by Indians (150 per 100 000) and Malays (30 per 100 000) (Lee & Khir 2007). Therefore, the understanding of biological and mechanical properties of bone will help in developing better orthopedic treatments. Technological research has looked forward for synthesis of new substituting biomaterials that mimic biological bone tissue (Kisailus 2016).

Calcium phosphate and bioactive glass have a potential for biomedical applications and showed promising bioactive features. For example, calcium phosphates (CaP) have some characteristics which is biocompatible, osteoconductive, and possess remarkable ability to interact directly to bone. In particular hydroxyapatite (HA), one of biomaterial that has high potential in dental and orthopedic applications due to its similarity to the mineral constituents of bone and teeth in the human body (Allo et al. 2012).

For many years, HA has been considered an important inorganic biomaterial which has attracted the attention of researchers related to biomaterials field due to its potential to stimulate optimal bone tissue regeneration (Wilcock et al. 2017). However, researchers have then realized that human biological bone is not solely HA with respect to its chemical composition, percentage crystallinity and crystal structure (Kulanthaivel et al. 2015). Besides that, when exposed in direct contact with biological system, HA resulted in extremely slow *in vivo* degradation and bioresorption rate to regenerate new bone tissue. This has limits its applications for orthopedic implants clinically (Kamitakahara et al. 2015). In addition, HA has also shown low mechanical properties particularly in terms of its tensile strength and fracture toughness (González Ocampo et al. 2016). For the aforementioned limitations, currently, HA can only used for non-loadbearing bone substitutes applications.

Furthermore, HA properties can be modified to make it suitable for broader biomedical applications by substitutions new element. The variety of substitutions can be introduced into the HA lattice by anions, cations, and functional groups. Among the ions that have been recommended to improve the HA properties are like carbon, cobalt, strontium, zinc and copper. These substitutions can give big impact toward properties of HA and also act as a tailor to modify physical, chemical, mechanical, and biological properties of HA (Kramer et al. 2014). These elements are could play the important role towards improving cell-material interactions of HA (Mardziah et al. 2009).

Carbonated hydroxyapatite (CHA) is one of example could enhance the properties of HA which carbonate groups introduced into apatite structure. It was found that to have a much closer composition that mimic to the mineral in natural bone. Generally, the amount of carbonate content in natural bone about 2-8 wt% (L. T. Bang et al. 2014). Carbonate substituted into HA structure is of special interest because the carbonate has an impact on different pathologies of human tissues (L. T. Bang et al. 2014). In other words, CHA relatively shows a higher bioactivity than HA and the smaller particle size of CHA would certainly bring about better tissue-implant interactions (Othman et al. 2016). Carbonate ion can substitute at two sites in the apatite structure either hydroxyl or phosphate ions position that giving rise to A-type and B-type carbonated hydroxyapatite, respectively (Kovaleva et al. 2008).

The orthopedic application of synthetic HA commonly faces the two significant challenges. The first one is its poor osteogenic properties compared to the natural bone apatite and the second one is limited angiogenesis at the site of application of construct (Kulanthaivel et al. 2015). Besides introducing carbonate into the apatite structure, another elements also can incorporate in the synthetic CHA such as strontium, cobalt, magnesium, zinc and silicon in order to overcome the limitations of HA. Basically, besides the main ions of Ca^{2+}, OH^-, PO_4^{3-} and CO_3^{2-}, other minority of ion that consist in natural bone such as strontium, cobalt, copper, zinc, magnesium and silicon (Allmae et al. 2012). All the ions that consist in natural bone and play an important role in providing the specific properties that need for bone structure. For example, doping of cobalt and strontium in synthetic apatite can give impart on angiogenesis and osteogenesis properties, respectively (Kulanthaivel et al. 2015). The materials that introduced as a substitution element should have some characteristics such as not induce to any cytotoxicity, immunological reactions, and inflammatory responses from the body (Poinern et al. 2014).

Generally, there are various synthesis routes that have been used to synthesize HA and CHA and among others include hydrothermal, sol-gel,precipitation, mechano-chemical, mechanical activation and nanoemulsion method (Othman et al. 2016). Commonly, A-type CHA was prepared by exposing HA at high temperature under flow of carbon dioxide. On other hand, B-type CHA was performed using wet method from precipitation reaction in aqueous media and also control the parameters such as pH, temperature and reagent concentration (Wu et al. 2009).

1.2 Problem Statements

Bone typically contains about 70% inorganic mineralized CaP phase and the remaining 30% mostly comprised of organic non-mineralized collagen matrix (Bose et al. 2013). Among the biomaterials used in orthopedic treatment is CaP based. These materials possess bioresorbable properties and they are able to facilitate new bone formation by allowing the migration, attachment, and proliferation of bone-forming cells (Bose et al. 2013). Therefore, no surprise if some of the most successful synthetic materials used in orthopedic implant are CaP based, particularly Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), (HA). HA-based materials are attractive ceramics for dental and biomedical applications since it is considered as the main mineral constituent in human bones and teeth (Yoruc et al. 2013). In addition, its physicochemical properties, solubility and crystallographic structure, are directly related to the mineralization processes in biological systems (Brundavanam et al. 2013). Besides that, HA has the unique characteristics such as biocompatible, non-toxic, non- immunogenic agent, non-inflammatory and also bioactive which suitable for biological systems (Rujitanapanich et al. 2014).

However, HA-based material has some disadvantages compared to biogenic apatite which is its resorption *in vivo* and too sluggish to induce a massive formation of a new bone tissue which considered as a serious drawback (Kovaleva et al. 2008). Besides that, HA also has poor mechanical properties in terms of tensile strength and toughness. Its application for HA-based material is limited to human body parts to those that require little or no load-bearing parts (González Ocampo et al. 2016). Due to its diverse applications, the materials properties accordingly need to be tailored for real world application. Hence researchers have tried to customize its properties such as bioactivity, mechanical strength, solubility and sinterability by controlling its composition, morphology and particle size (Agrawal et al. 2011).

Improvement of biological and physicochemical properties of HA can be achieved by introducing with new elements in apatite structure that are usually present in natural apatites of bone. Non-stoichiometric HA has a hexagonal crystal structure that enables some ionic substitutions according to charge type, charge size and ionic radii (Uysal et al. 2014). Most natural apatites are non-stoichiometric because of the presence of minor constituents such as cations ($Mg^{2+,}Mn^{2+}$, Zn^{2+},Na^{+}, $Sr2^{+}$ and Co^{2+}) or anions (HPO_4^{2-} or CO_3^{2-}) (Bose et al. 2013). These ionic substitutions can affect the surface charge, lattice parameters, crystallinity and morphology which can result in changes in thermal stability, mechanical properties, solubility and bioactivity of HA (Kumar et al. 2012).

The past few decades, much research has demonstrated that a variety of ionic substitutions that can be incorporated into synthetic HA to produce a mineral composition more mimic to the natural bone tissue (Ratnayake et al. 2016). Numerous research works have focused on the synthesis of HA biomaterial substituted with single or multi-ion substitution of HA (L T Bang et al. 2014). For example, the substitution of small amounts of cobalt into the HA lattice which give impart to angiogenesis properties of HA. However, the performance of the cobalt doped hydroxyapatite needs to be confirmed in an *in vivo* model to prove the function of cobalt as tools to improve tissue regeneration (Kulanthaivel et al. 2015). Besides that, another ion such as strontium also able to introduce in HA lattice. Strontium is considered as a bone-seeking element that can provides a beneficial effect on bone growth (Aina et al. 2012). Moreover, Sr substituted in HA lattice will served a good properties including higher compressive strength, good workability and low cytotoxicity (Guo et al. 2005).

Basically, the natural bone which differs from pure HA contains about 4–8 wt% of carbonate with several multi-substituted ions such as strontium, cobalt, magnesium and zinc in its structure (L T Bang et al. 2014). Carbonated hydroxyapatite (CHA) is considered as a promising alternative to HA-based bone substitutes in order to mimic that of the natural bone. (Wong & Noor 2016). Commonly, CHA has been widely used for bone tissue engineering (BTE) due to its great biocompatibility, biodegradability and osteoconductivity (Guo et al. 2013). Despite their excellence properties, CHA-based materials suffer from low thermal stability. The decomposition of CHA-based material was reported to begin at about 800˚C in normal sintering atmosphere (air) (Baba Ismail & Mohd Noor 2011). Generally, higher sintering temperature is usually required to produce highly densified products in order to improve mechanical properties. Thus, the proper sintering is required as poor control of heat treatment would result in CO_3 loss, leading to partial or total decomposition and hence would affect the physical and mechanical properties of the synthetic materials (Wong & Noor 2016).

One way to improve in terms of bone regeneration is by altering the chemical composition of HA in a controlled manner through multi-doping of the ions such as carbonate, magnesium, strontium, cobalt and zinc. New elements such as strontium and cobalt ions are also able to introduce into the carbonated hydroxyapatite structure based on trace elements that constituent in bone structure in order to improve their biological and mechanical properties. The presences of these ions are expected to stimulate rapid osteogenesis and angiogenesis, which is the key requirement for successful bone regeneration.

Commonly, CHA-based material was synthesized from two synthesis routes such as the precipitation method and nanoemulsion method. One of the most widely used methods is nanoemulsion, where chemical reactions take place between calcium and phosphorus ions under a controlled pH and temperature of the solution. The nanoemulsion method has been found that promising for the synthesis of nanosize particles, B-type CHA formation in the spherical shape and single phase which similar reported by Zhou et al. (2008).

In the last four decades, important advances have been made in the improvement of scaffolds for biomedical applications (Dhandayuthapani et al. 2011). Nowadays, most scaffolds development involved the production of ceramic foams by coating a polymeric sponge with a bioceramic slurry (Bellucci et al. 2011). Using this replication method for tissue engineering, scaffolds may achieve a significantly richer porosity, usually exceeding 80%. However, abundant of porosity is associated to a very high brittleness which destabilizes the manageability of the scaffold surface and it also would affect the mechanical properties of the material. (Bellucci et al. 2011). Therefore, fabrication of dense product of biomaterial will be improved in term of mechanical properties that can overcome the limitation of implant to human body parts to those that only require little or no load-bearing parts.

In term of bioactivity, one of main characteristic that required as a biomaterial is bioactive which ability to interact with tissue in human bone (Patel & Gohil 2012). Commonly, the bone-bonding ability of a material is often evaluated by *in vitro* bioactivity evaluation which the ability of apatite to form on its surface of the sample in a simulated body fluid (SBF) solution (Kokubo & Takadama 2006). Normally, the materials will be soaked in SBF solution for 7, 14 and 28 days and it has been described by many researchers (Bellucci et al. 2011); (Kramer et al. 2014); (Saber-Samandari et al. 2016). The formation of the apatite layer on the surface will provide beneficial effect on cell adhesion during implantation. Thus, this method can assist in the efficient development of new types of bioactive materials.

Therefore, the aims of this work are to produce and optimize the composition of a range of multi-doped carbonated hydroxyapatite (CHA) powders using nanoemulsion method followed by the fabrication and sintering of dense multi-doped CHA products in the form of a pellet. The introduced of wet CO_2 after sintering will be act as aids to compensate the carbonate loss due to decomposition during sintering which could further improve the mechanical properties of final products.

1.3 Research Objectives

The main objectives of this work are:

1. To produce a range of cobalt and strontium as co-substituted CHA powders (multi-doped CHA) by nanoemulsion technique.
2. To optimize the composition as-synthesized multi-doped powders (Co-Sr) CHA based on chemical and physical characteristic.
3. To fabricate and characterize dense multi-doped CHA.
4. To investigate *in vitro* bioactivity and mechanical properties of dense multi-doped CHA.

1.4 Scope of works

In general, this work will be divided into four main stages. Synthesis of CHA and multi-doped CHA powders were done in the first stage of this work, followed by characterization and optimize as-synthesized multi-doped CHA powders. The final parts of this work was fabricated of dense multi-doped CHA in the form of pellet via dry pressing method, which then sintered at 900°C CHA. Then, characterization of sintered dense multi-doped CHA in terms of chemical, physical, bioactivity and mechanical properties. Figure 1.1 shows flowchart of the research work.

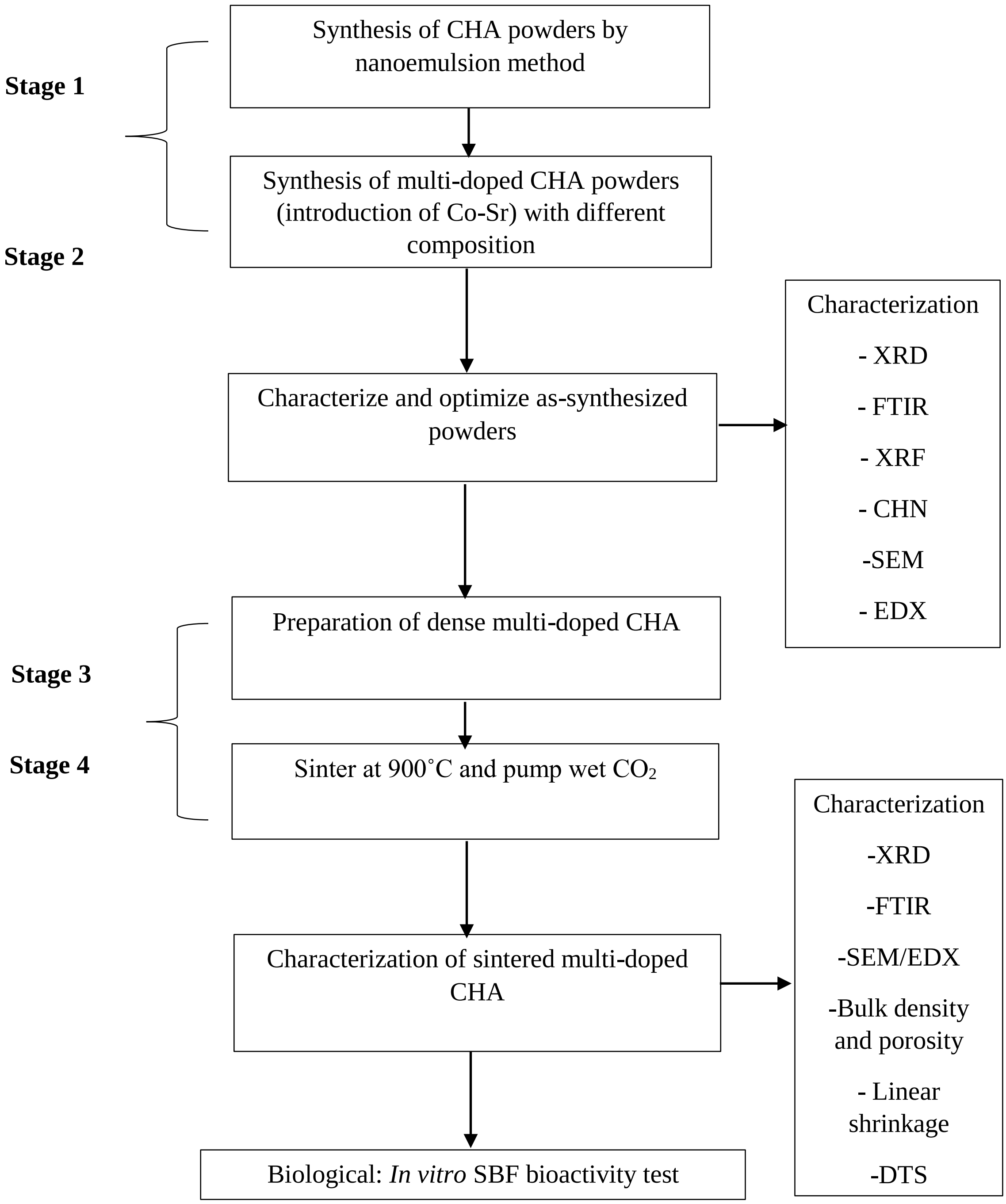

Figure 1.1: Flowchart of the research work

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Worldwide, bone injury or fracture is considered as a major health issue. Therefore there have been extensive investigations into the fracture behavior of human bone. Bone fracture is among of the expensive surgery for medical applications. In nature, bone has some characteristics such as good structure, lightweight and yet tough (Kataruka et al. 2017). However, the fracture properties of human bone beneath fall-like loading conditions remains poorly documented (Gauthier et al. 2017). Fractures can happen in variety ways such as trauma, cancerous, total hip revisions and metabolism (Umadevi & Geethalakshmi 2011). Thus, bone substitutes were become important in bone surgery due to highly demands overcome bone fracture issues over the last few decades.

Bone grafting is a surgical procedure which replacement of bone fracture with the material from either patient's own body (autograft), donor (allograft) or artificial bone (Teresa Mao 2013). However, both of bone graft techniques have drawback that needs to be considered. Generally, autograft is standard technique that used for the surgical procedure. However, it has some limitations such as pain, donor site morbidity and long period time of operation (Kheirallah & Almeshaly 2016). For allograft technique, its look to overcome issues that related to autograft due to allograft shows better results which allow a faster recovery and without involved donor site morbidity (Rodríguez et al. 2015). Unfortunately, allograft not provided osteogenic properties which it might completely interact with host tissue. Another type of bone graft which is substitute materials could not suffer both osteoinductive and osteogenic properties and mechanical properties.

In recently, biomaterials have achieved increasing interest for regenerate and replacement of tissues in human body. It is proved by developing of ceramic material as a biomaterial to replace lost tissue or organ structure (Adrezin 2004). Hydroxyapatite (HA) showed the great potential as a biomaterial for bone substitution due to its characteristics that closely similar to natural bone such as biocompatibility, bioactivity and osteoconductivity (Orlovskii et al. 2002). However, further studies showed that composition of HA differs to human bone and another ion like carbonate which traces in HA lattice in a range 2-8wt% (Kee et al. 2013). Thus, there have been drive for researchers to investigate more about carbonated hydroxyapatite (CHA) which shows better bioresorbability and enhances osteointegration rate. Moreover, other elements also were introduced in CHA and these substitutions could enhance the properties with many aspects such as chemical, physical and biological response of the bone apatite (Kulanthaivel et al. 2015).

In general, the aims of this review are to provide fundamental understanding and overview of the structure, function and properties of natural bone tissues in human body. Then, it followed by the information about bone grafts. The review then more focusing toward biomaterial and bioceramic material for example calcium phosphate based bioceramics, hydroxyapatite and carbonated hydroxyapatite. This will then followed by the review about new elements as ion substitutions that possible introduced into HA structure. Lastly, the final part of this review then described densification of ceramic powder in order to produce highly densified multi-doped CHA in this study.

2.2 Bone Anatomy

Human skeletal system is the system of bones that related with cartilages and joints of human body. Moreover, the skeleton is definite as the hard framework of human body around which the entire body is supported. Mostly all the hard parts of human body are components of human skeletal system (Umadevi & Geethalakshmi 2011). In addition, skeleton that allow the body movement and protects internal organs (Driessens & Verbeeck 1990). Based on the Table 2.1, it showed some of important functions of human skeleton.

Table 2.1: Important functions of human skeleton (Umadevi & Geethalakshmi 2011)

Functions	**Explanation**
Strength, support and shape	The skeleton will gives strength, support and shape to the body.
Protection of soft organ	The skeleton will be protects inner soft and sensitive organs like heart and brain from external shocks.
Leverage for movements	The skeleton in all parts of body were attached with muscle and helps for movements of body parts
Production of red blood cells	Bones have hemopoeitic activity (blood cells production)

In human body, the skeleton is composed of three components which are bones, cartilages and joints. Basically, bone is a tough and rigid form of connective tissue in human body. From biologically perspective, bone is a dense type of connective tissue saturated with inorganic salts mainly the salts of calcium which are calcium phosphate and calcium carbonate (Umadevi & Geethalakshmi 2011). From engineering in point of view, bone is a natural composite material consists of 65-70% of inorganic mineralized matrix and the remaining 30-35% of organic mineralized matrix, which predominantly collagen type I (Zaslansky et al. 2006). Moreover, inorganic salts which content of the salts of calcium are essentially responsible for rigidity and hardness which will make bone resist to compression caused by the forces of weight and impact. Organic connective tissue portion of the bone makes it resilient and bone can afford resistance to tensile forces.

2.2.1 Bone structure

Bone has important functions in the human body such as locomotion, support and protection of soft tissues, calcium and phosphate storage, and sheltering of bone marrow (Florencio-Silva et al. 2015). It also act as component that important to protect inner soft and sensitive organs like heart and brain from external shocks. In the aspect of mechanical properties, different kind of bones having different mechanical properties and it similar reported by Currey (2004). In addition, bone also act as weight bearing organ of human body and take responsibility for almost all strength of human skeleton. Based on Figure 2.1, bones can be classified into five different types of bones such as long bones which normally found on arms, legs, hands, and feet. Besides that, short bones which found at the wrist and ankles while flat bones have found at ribs, shoulder blades and hip bones. Lastly, irregular bones found in the facial bones and sesamoid bones at special short bones and patella.

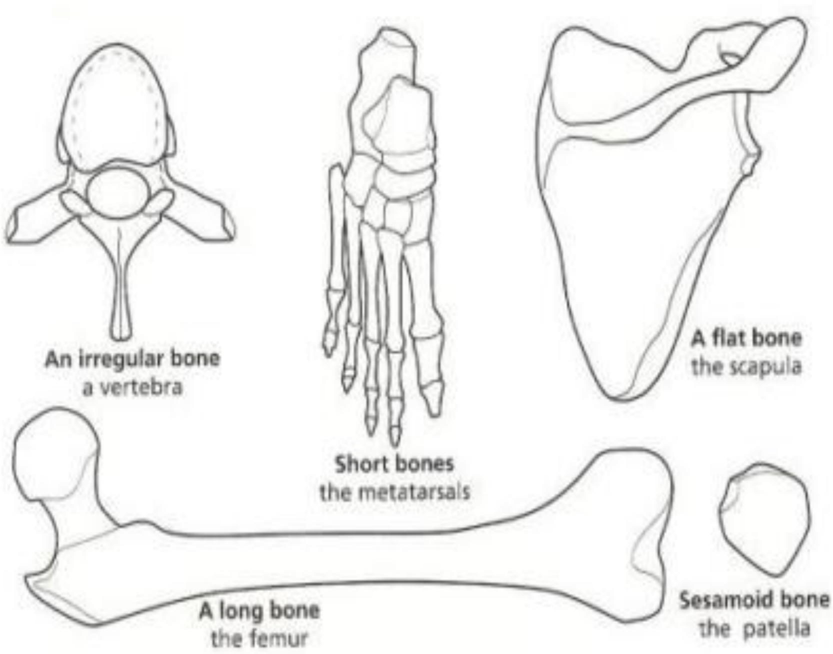

Figure 2.1: Bone Classification According to Shape (Umadevi & Geethalakshmi 2011)

Generally, compact bone and spongy bone are the two kinds of bone tissue that form in bones. Based on Figure 2.2, the microscopic showed that two types of bone tissue which are compact bone and spongy bone. Basically, compact bone normally most composed on outer layer while spongy bone in inner layer. Compact bone is the structure that forms with high mechanical properties like tough it consist in the majority of bones. Due to the better mechanical properties of compact bone, the main functions of this type is to support the entire body if compared to spongy bone (Nganvongpanit et al. 2015). For spongy bone, this bone is porous and due to their characteristic of porous nature helps to hold the bone marrow and soft tissue that produces red blood cells (Capuani et al. 2007).

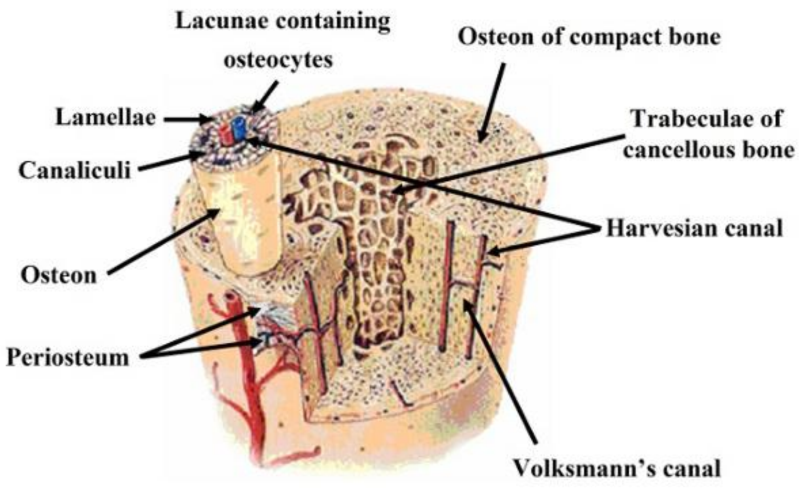

Figure 2.2: Schematic diagram of compact bone and spongy (cancellous bone). (Umadevi & Geethalakshmi 2011)

2.2.2 Bone Composition

Bone consists of organic collagen fibers and inorganic mineralized matrix bone mineral in the form of small crystals of apatite. In bone, the percentages of inorganic mineralized matrix is approximately 60-70% and most of the rest is proteinaceous material, which predominantly collagen type I (Widyastuti 2009). Another journal also was reported similar results which the bone actually has composed of the main mineral (50-60wt%), collagen (30-40 wt%) and water (10-20 wt%) (Mirzaali et al. 2016). The quantitative composition of bone mineral is complex and can vary within one bone, between bones, between individuals, between species, with diet/with age and with pathological conditions (Boskey 2013). Moreover, the amount bone mineral constituent, proper arrangement and characteristics of each of bone mineral based on quantity and quality will be define the properties of bone. The composition of bone mineral is shown in Table 2.2.

Table 2.2: Composition of the mineral phase of bone in comparison to stoichiometric HA (Allmae et al. 2012)

Composition	**Bone mineral (wt%)**	**Stoichiometric HA (wt%)**
Calcium (Ca)	34.80-36.60	39.6
Phosphorus (P)	15.20-17.10	18.5
Carbonates (CO_3)	4.80-7.40	
Sodium (Na)	0.90-1.00	
Magnesium (Mg)	0.60-0.72	
Chlorine (Cl)	0.10-0.13	
Fluorine (F)	0.03-0.10	
Potassium (K)	0.03-0.07	
Strontium (Sr)	0-0.05	
Silicon (Si)	0-0.05	
Zinc (Zn)	$0\text{-}3.9\times 10^{-3}$	
Chromium (Cr)	$0\text{-}3.3\times 10^{-5}$	
Cobalt (Co)	$0\text{-}2.5\times 10^{-6}$	
Manganese (Mn)	$0\text{-}1.7\times 10^{-5}$	

The contents of bone mineral that consist in the bones are presented in Table 2.2. Hydroxyapatite (HA) has been described as the synthetic material which important component of the bone mineral of bone and it has been demonstrated more than 60 years ago using X-ray diffraction (Boskey 2013). Generally, HA is main constituents of inorganic part that similar crystal structure to the natural bone mineral and it can be found in hard tissues such as teeth and bones (González Ocampo et al. 2016). However, chemical composition of bone crystals did not correspond to the chemical composition of HA (Rey et al. 2010). Further studies was directly found that stoichiometric HA differ with natural bone due to lack in carbonate ions (Baba Ismail & Mohd Noor 2011). The amount of carbonate ions in bone mineral in a range from 2% to 8% and it depending on the individual's age (Kee et al. 2013) . Besides the main ions of $Ca^{2+,}$ OH^{-}, PO_4^{3-} and CO_3^{2-}, other minority of ion that consist in natural bone such as strontium, cobalt, copper, zinc and magnesium (Allmae et al. 2012). All the ions that consist in natural bone based on Table 2.2 play an important role in providing the specific properties that need for bone structure.

2.3 Bone graft

Generally, human skeletons will be provided a strength, help for movement, support and shape to the human body as mentioned earlier. It also will be protected inner soft and sensitive organs like heart. However, human skeletons have potential to susceptible for bone injury or bone defect caused by many different ways, which trauma, infections, tumors and osteomyelitis (Han et al. 2017). Fracture occurs in bone are related to the quality of bone which it influenced by mechanical behavior, biological factor and the microstructure (Kataruka et al. 2017). Besides that, bone resorption is one of the bone defect which natural phenomenon happen due to aging (Teresa Mao & Kamakshi V 2013); . Thus, bone graft has been developed to overcome bone defect issues and become standard surgery for repair damaged hard tissues.

A bone graft is a surgical procedure that mostly used to fix problems with relating to bones or joints resulting from trauma or problem joints (Teresa Mao & Kamakshi V 2013). In another word, bone graft is an alternative way to replace of missing bone that use material from either patient's own body (autograft), donor (allograft) or man-made bone graft. This technique is possible because the bone tissue has ability to regenerate completely at part that it has to develop (Teresa Mao & Kamakshi V 2013); (Widyastuti 2009). Generally, graft material is designed to replace the broken bone and it will be resulting in a completely incorporated region of new bone in human body. In fact, 3.5 million of bone graft procedures performed every year around the world (Kheirallah & Almeshaly 2016).

Table 2.3: Four important characteristics required for bone grafts (Kheirallah & Almeshaly 2016); (Hosokawa 2013)

Characteristic	**Explanation**
Osteoconductive	Ability to support bone growth on a surgical part, during which pores, channels, and blood-vessels are formed within bone
Osteoinductive	Stimulation of osteoprogenitor cells to differentiate into osteoblasts then begin new bone formation.
Osteointegrative	Ability to direct contact of living bone to graft material
Osteogenesis	Provide formation of new bone by osteoblasts within the graft material

Table 2.3, it showed four important characteristics the required for bone grafts. For a long time, autograft and allograft have been considered as the best treatments for bone substitutions since both methods provide a fast osteointegration with the surrounding tissues after implantation in human body (Bellucci et al. 2011). This explain why, autografts and allografts are common techniques that widely used to repair bone injuries, trauma and deformities. Currently, autograft is the best standard bone repair clinically, it could encourage rapid as osteoinductive and do not pose a risk of disease transmission. Nevertheless, there are some disadvantageous of using autograft technique which are donor shortage, surgical trauma, increased operative time, pain, infection,

scarring, blood loss and donor site morbidity (Kheirallah & Almeshaly 2016); (Wei et al. 2015).

On the other hand, allograft is bone donated from another member of the same species which have been increasingly applied in clinical treatment in the past decade as an alternative to autograft. The advantages of allograft are wide availability in various shapes and sizes, avoidance of donor-site morbidity and allow fast recovery if compared to autograft (Rodríguez et al. 2015). However, allograft has some limitations which are immune rejection, delayed incorporation, poor stability and risk of disease transmission (Kheirallah & Almeshaly 2016). Both types of bone graft techniques have several disadvantages and could affect the human body.

Thus, bone graft substitutes (BGS) have been introduced in the past few decades to overcome the limitations of both type of bone graft for treatment of bone defects (Wagner-Ecker et al. 2013). In order to the limitations of these techniques, bone graft substitutes (BGS) shown positive results which it has potential alternative for supporting the newly formed bone tissue (Bellucci et al. 2011). Moreover, this technique has some advantages because of their unlimited availability, no risk of disease transmission and flexible in terms of composition without batch variability. Unfortunately, they in lack osteogenic and angiogenic properties (Kheirallah & Almeshaly 2016).

2.4 Biomaterials

Researchers are still facing forward regarding bone defect repair embody normal procedures of autografts and allografts**.** However, both processing techniques are potentially reduce the osteoinductivity, osteoconductivity, and mechanical strength of the graft (Allo et al. 2012). To overcome these limitations, development of biomaterials for bone graft substitutes (BGS) that can repair or regenerate bone is highly needed. Past five decades, biomaterials were introduced and still in progress in this field to improved biomaterials that suitable for biomedical applications (Mediaswanti et al. 2013).

In history, the first generation of biomaterials was developed to mimic tissue of human bone due to physical damage and disease during the 1960s until 1970s. Then in the 1990s until 2000s, the researchers were introduced material as biomaterials that can interact and matched with biological system in human bone. Lastly in the 2000s, materials that have been combined both characteristics which are bioactive and resorbable properties as biomaterials and it called as the third generation of biomaterials (Ning et al. 2016). The history of biomaterials shown in Figure 2.3.

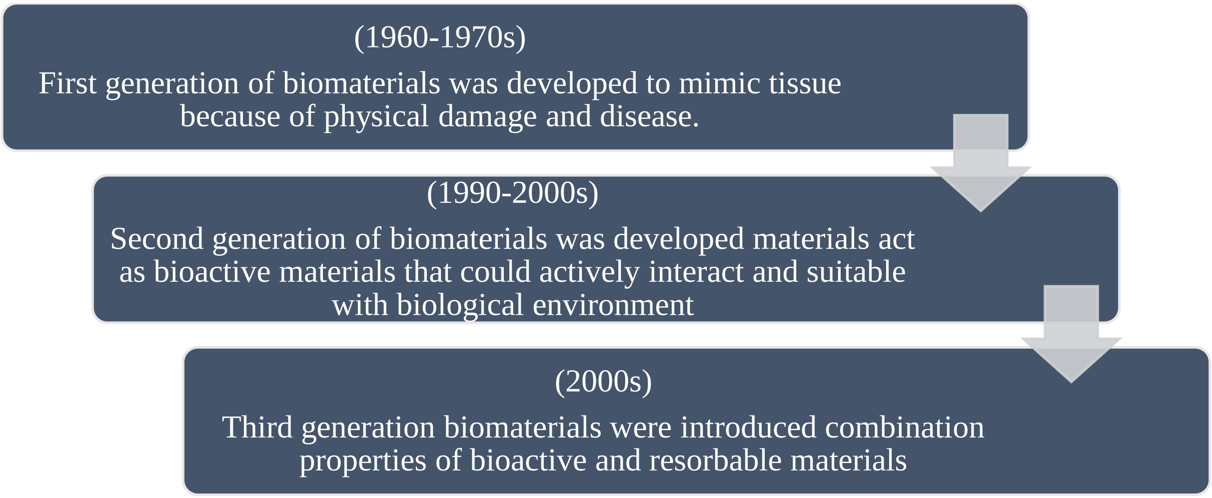

Figure 2.3: Flowchart of development and evolution of three generations of biomaterials (Ning et al. 2016)

Based on the reaction of the tissue to biomaterial in Table 2.4, it can be classified into four categories which are toxic material, bioactive material, bioinert material and bioresorbable material.

Table 2.4: Three categories of reaction between tissue and biomaterial (Anusavice 2003)

Classification	Tissue Response
Toxic material	Tissue dies
Bioinert material	Tissue form an adherent fibrous capsule around the implant
Bioactive material	Tissue for interfacial bond with implant
Bioresorbable material	Tissue eventually replace implant as new bone formation take place

For biomedical applications, the most important criteria which need to be considered are the materials used for implant should be biocompatible, bioactive, and biomechanical compatible. In general, biomaterials are materials that will not cause negative response on the tissue after implantation and non-cytotoxic (Mediaswanti et al. 2013). Nowadays, in implant applications were developed the combination of both properties which are bioactive and resorbable materials that have the ability to help the body to recover itself post-implantation (Mediaswanti et al. 2013). In other word, biomaterials used for implant applications should have some important properties that mentioned earlier in order to long-term usage without rejection in human body. In addition, other factors such as material used, load applied during function, patient well-being age and technique that used are also important in determining the adaptation and endurance of the biomaterial (Anusavice 2003). Besides, the design and selection of biomaterials need to be consider based on situation and condition physical damage or disease of that particular patient (Patel & Gohil 2012). Based on Figure 2.4, it shown that development of biomaterials made up from any substance or combination of material except than synthetic or natural in origin, which replaces partially or totally any tissue, organ or function of the human body and it can be used for longer period of time without giving any negative effect for the body. The aim for introduced biomaterials as the best alternative way for biomedical applications to maintain or improve the quality of life of the individual.

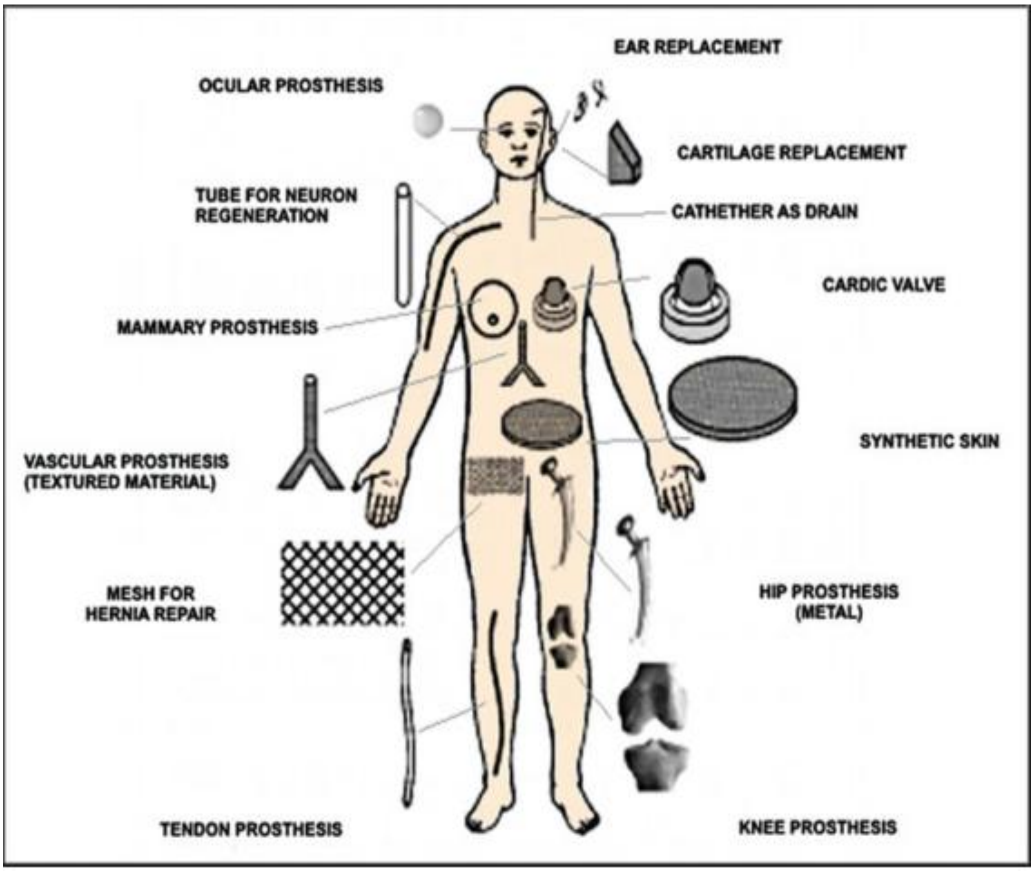

Figure 2.4: Examples of biomedical implants (Anusavice 2003)

2.5 Bioceramics

In general, bioceramics are referred to biocompatible ceramic materials that always applicable for biomedical or dental applications (Nasseh A. 2009). In another word, ceramic biomaterials are used for repair and replacement of diseased and damaged parts of musculoskeletal systems (Adrezin 2004). Moreover, ceramics are widely used compared to metals due to biological inertness of ceramic as biomaterials for medicine and dentistry for the past three decades (Thamaraiselvi & Rajeswari 2004). Nowadays, bioceramics are widely used for medical applications such as largely for implants in orthopaedics, maxillofacial surgery and for dental implants. Three basic types that are related to bioceramics which are bioinert high strength ceramics, bioactive ceramics which form direct chemical bonds with bone or even with soft tissue of a living organism (Thamaraiselvi & Rajeswari 2004). As example, Alumina (Al_2O_3), Zirconia (ZrO_2) and carbon are related to bioinert characteristic while bioglass and glass ceramics are bioactive. Lastly, the bioresorbable characteristic is referred to the material that can dissolve or degrade its own within the body with predetermined rate or by controllable manner (Popov et al. 2014). The example of the bioresorbable material such as calcium phosphate ceramics that widely used as bone substitute materials.

In addition, bioceramic materials are processed to obtain one of four general types of surfaces and associated mechanisms of tissue attachment (Kohn and Ducheyne, 1992). First, fully dense relatively to inert crystalline ceramics that attach to tissue. Second, porous comparatively to inert ceramics into which tissue ingrowth occurs and creating a mechanical attachment Next, fully dense, surface-active ceramics that attach to tissue via a chemical bond. Lastly, resorbable ceramics that incorporated with tissue and eventually are replaced by host tissue (Adrezin 2004).

In general, most common bioceramic materials that widely have been used for biomedical applications such as alumina, stabilized zirconia (Y-TZP, Y-stabilized Tetragonal Zirconia Polycrystal), and calcium phosphates-based materials, in particularly hydroxyapatite (HA) (Heimann 2002). Carbonated hydroxyapatite (CHA) is one example of bioceramic materials that recently has been utilized in tissue engineering (TE) applications. The basic functions of CHA as biomaterial are to assist in healing defects, to correct abnormalities and to improve function of body. CHA also play important roles in order to repair and reconstruction of damaged and worn out part of the human body or