CHAPTER II

LITERATURE REVIEWS

2.1 Bone Structure

Bone is a living tissue that makes up the body's skeleton. The materials making up bone form a composite material formed by both organic and inorganic phases. The organic part (22%) of the matrix is collagen. This is made in tracellular bone as tropocollagen and then is exported it associating into fibrils [10]. Collagen is a flexible tough protein that dictates the shape of the bone. The inorganic part (69%) consists mainly of crystalline mineral, salt and calcium, which is present in the form of apatite (Table 2.1). The apatite crystals in bone structure are formed as slender needles or plates, 20-40 nm in length by 1.5-3 nm in thickness, in the collagen fiber matrix as shown in Figure 2.1. Apatite is a group of calcium phosphate salts that takes part in composition of the bone and in nature's evaluation of the decomposition process occurring in minerals bony tissues. It has been reported that the decomposition process of mineral tissue with time has been tested on human bones aged over one century [11]. When these bones were x-rayed, it revealed the presence of such compounds as $Ca_4P_2O_9$, α- $Ca_3(PO_4)_2$ and β- $Ca_3(PO_4)_2$, as shown in Figure 2.2. This figure suggests that the natural decomposition of bone takes place according to the following reactions:

$$Ca_{10}(PO_4)_6(OH)_2 \longrightarrow 2Ca_2P_2O_7 + Ca_3(PO_4)_2 + 3CaO + H_2O \quad \text{.....(2.1)}$$

$$Ca_{10}(PO_4)_6(OH)_2 \longrightarrow 3Ca_3(PO_4)_2 + CaO + H_2O \quad \text{.....(2.2)}$$

$$2Ca_2P_2O_7 + 2CaO \longrightarrow Ca_4P_2O_9 \quad \text{.....(2.3)}$$

There are two types of bone, compact and sponge bone. The cortical bone, also known as compact bone which is one of two main types of osseous tissues. Cortical bone is dense and forms the surface of bones, contributing 80% of the weight of a

human skeleton. It is extremely hard, formed of multiple stacked layers with few gaps. The cancellous bone also known as trabecular or sponge bone is the type of osseous tissue with low density and strength but very high surface area that fills the inner cavity of long bones. Its main function is to support the body, protect organs, provide levers for movement, and store minerals. The external part of cancellous bone contains red bone narrow where the production of the blood cellular component takes place. Bone has an internal mesh like structure, and its density may vary at different points. Although the properties of bone vary from point to point and the proportion of the diverse substances varies according to the different parts of the skeleton and bone tissue it contains (Table 2.2). Therefore, it is feasible to design the porosity of substitute materials to be similar to that of trabecular bone which has 50-95% porosity and a network of interconnected pores.

Table 2.1 Composition of bone [10]

Components	Amount (wt.%)
Mineral (apatite)	69
Organic matrix	22
Collagen	(90-96% of organic matrix)
Others	(4-10% of organic matrix)
Water	9

Table 2.2 Physical characteristics of bone [11]

	Cortical bone	Cancellous bone
Density (g/cm^3)	1.6-2.1	-
Compressive strength (MPa)		2-12
Bending strength (MPa) (after testing in ringer's solution)	50-150	-
Young's modulus (GPa)	7-25	0.05-0.5
Fracture toughness, K_{IC} (MPa/m^2)	2-12	-

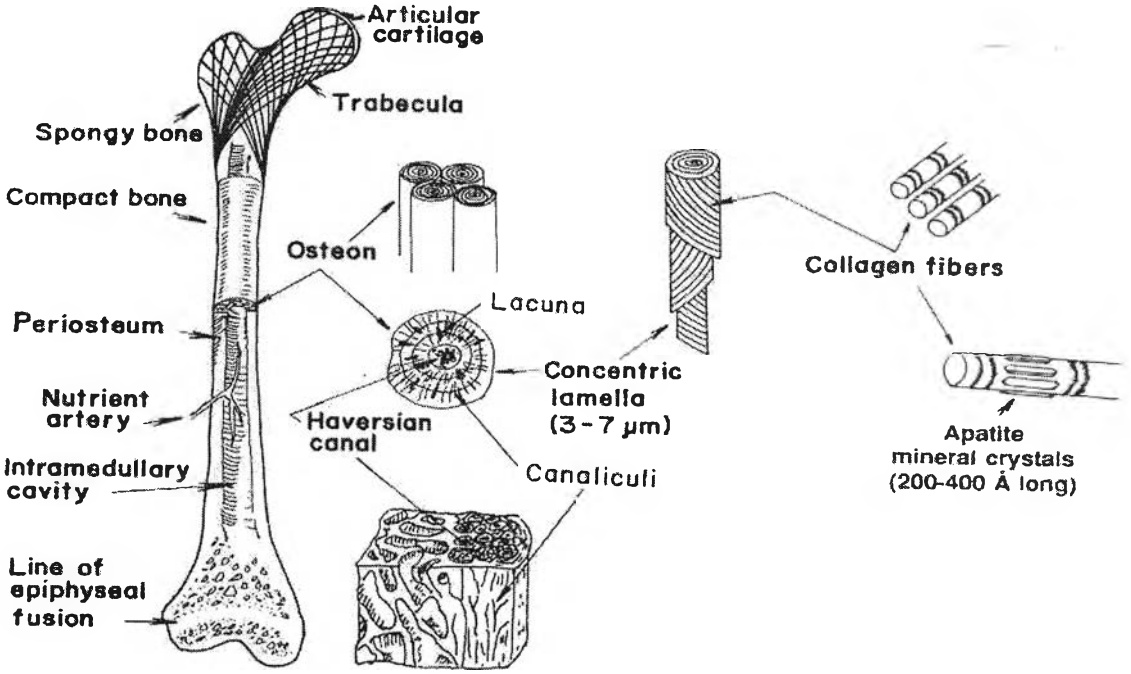

Figure 2.1 Organization of a typical bone [10].

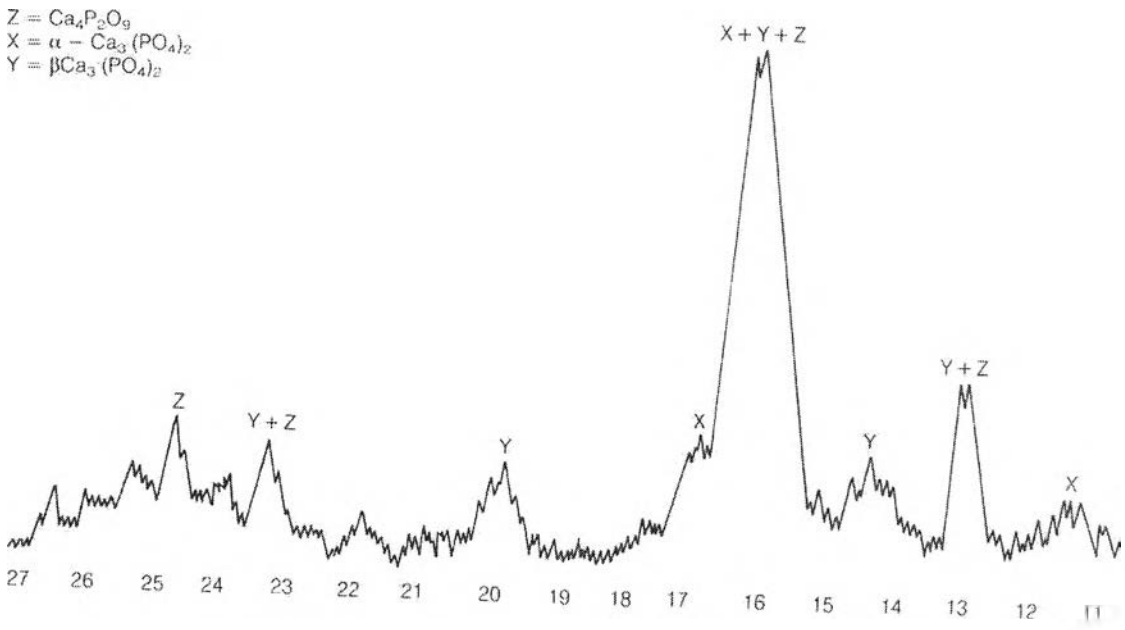

Figure 2.2 Typical X-ray analysis of a human bone [11].

2.2 Biomaterials and Bone Replacements

Bone repair and regeneration of defects arising from trauma, tumor or bone diseases display a complex and serious clinical problem in orthopedic surgery. Some of the therapies employed in order to solve these problems are use of autograft, allograft, and artificial implants; each type has its advantage and disadvantage. Autograft is a popular procedure for reconstructive surgery because it is taken from the patient. However, the amount of transplant is usually limited in supply, the resistance of pain, the nerve damage and possible infection. Allografts to extend the graft volume are used in current clinical practice, though these pose a risk of transmission of bacterial and viral disease and legal; religious and cultural limitations are also problematic [11]. Therefore, some of the therapies were employed in order to solve these problems including use of artificial implants for repair and reconstruction of damaged parts of the human. Artificial bone replacement materials made from metals, ceramics, polymers, and composite are of a great importance [4,11-13]. Many ceramic materials as biomaterials used for to repair and reconstruction of damaged parts of the human skeleton. Ceramics used for this purpose are termed bioceramics. Replacement of tissue has two alternatives; (i) transplantation and (ii) implantation. The significant advantages of bioceramic as implants over transplants are availability, reproducibility, reliability and the fact that they do not pose any viral or bacterial risk of patients. Table 2.3 presents the ceramic materials as biomaterials which are used in medical applications [10].

Table 2.3 Present uses of bioceramics in medical applications [10]

Applications	Materials	Applications	Materials
Orthopedic load-bearing applications	Al_2O_3	Temporary bone space fillers	TCP
	Stabilized zirconia		Calcium and phosphate salts
	PE-HA composite	Periodontal pocket obliteration	HA
Coating for chemical bonding (orthopedic, dental, and maxillofacial prosthetics)	HA		HA-PLA composite
	Bioactive glasses		TCP
	Bioactive glasses		Calcium and phosphate salts
Dental implants	Al_2O_3	Maxillofacial reconstruction	Al_2O_3
	HA		HA
	Bioactive glasses		HA-PLA composite
Alveolar ridge augmentations	Al_2O_3		Bioactive glasses
	HA	Percutaneous access devices	Bioactive glasses-ceramics
	HA-autogenous bone composite		Bioactive glasses
	Ha-PLA composite		HA
	Bioactive glasses	Orthopedic fixation devices	PLA-carbon fibers
Otolaryngological	Al_2O_3		PLA-CaP based glass fibers
	HA	Spinal surgery	Bioactive glasses-ceramics
	Bioactive glasses		HA

From the Table 2.3, we found that advances in many specialty bioceramics such as alumina, zirconia, hydroxyapatite, tricalcium phosphate and bioactive glasses have made significant contribution to the development of modern health care industry and have improved the quality of human life. These are the ceramics, which can be used inside the body without rejection to augment or replace various diseased or damaged parts of the musculoskeletal system. The superior biocompatibility of calcium phosphate contributed by their compositional resemblance with the bone mineral has allowed them to be used for many applications inside the body. Based on the observed tissue response, biomaterials can be classified into four large groups, including nearly inert, porous ingrowth, bioactive and resorbable materials [11]. The various classifications of implant are detailed below, along with examples of typical medical ceramics. The relative bioactivity of each of four types of implant tissue reactions is shown in Figure 2.3 [14].

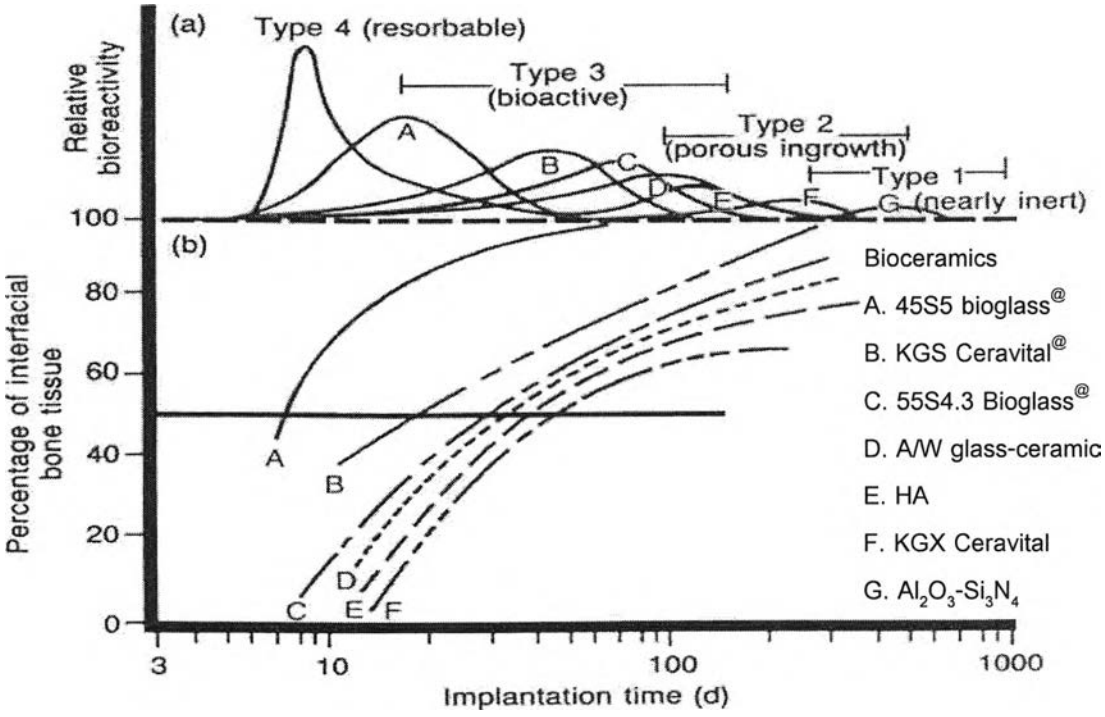

Figure 2.3 Bioactivity spectrums for various bioceramic implants (a) relative rate of bioreactivity and (b) time dependence of bone formation of bone bonding at an implant interface [1].

(i) Nearly Inert crystalline bioceramics: There is minimal interaction with the host tissues, the implant will be covered with non-adherent fibrous tissues as the host tries to isolate itself from the foreign body. Fixation is by pure mechanical interaction. This is also known as morphological fixation. Materials of this type include alumina (Al_2O_3) and zirconia (ZrO_2) and are used as femoral heads in artificial hip joints. Additionly, alumina, zirconia and its composites produce excellent mechanical properties [13,15,16]. Several researchers have successfully fabricated these materials for biomedical applications. These materials did not bond directly to bone nor allow bone ingrowth. Consequently, biocompatible materials such as HA, TCP and bioactive glass need to be coated onto the surface or incorporated as second phases [16-18]. For example, X. Miao et. al [9] improved the biological response of porous zirconia ceramic, with high mechanical strength as it was a bioinert material, by coating with hydroxyapatite - borosilicate glass. This result suggests that the porous zirconia was modified with a bioactive composite HA surface that was formed on the top of the material coating for biomedical applications.

(ii) Porous ingrowth: Fixation is achieved by biological ingrowth of the host tissues into the implant pores. This is also known as biological fixation such as is done with hydroxyapatite and hydroxyapatite coated porous metals used for femoral components of artificial hips [17,18]. In the porous form, HA can be colonized by bone tissue with the same characteristics as implanted tissues. For colonization of the pores to take place, they must be larger than 50-100 μm according to some authors [11,19,20,21]. For example, M.V Doernberg et. al. studied the effect of macropore size, with pore sizes of 150, 260, 510 and 1120 μm, of β-TCP blocks on the *in vivo* behavior of ceramic scaffolds that were implanted in metaphysical or epiphysial defects in sheep. It was found that all samples were found to be biocompatible, osteoconductive, and resulted in a fast turnover from ceramic to bone that might be more efficient in this animal model as bone substitute [22]. Therefore, the existence of the pores can be accompanied by a reduction in the mechanical stability of calcium phosphate based ceramics. This loss of stability is often cited as a restriction in the use of Ca-P ceramics in clinical practice [15,22,23]. A.C Queiroz et.al. have studied and prepared porous

hydroxyapatite for controlled drug delivery using the polymeric sponge method. It was found that the results produced have a pore size of 150-400 μm which is sufficient for bone growth and drug adsorption [24].

(iii) Bioactive materials: Fixation is achieved by chemical bonding between the implant and the host tissues such as calcium silicate based glasses, ceramic and glass ceramic composites [4,13,17,25]. They are used to fill defects in the jaw following the removal of teeth in dental surgery and as bone replacement for orthopaedic applications [7,26]. An *in vivo* study by U. Ripamonti demonstrated that the induction of bone took place in hydroxyapatite, with hydrothermally chemical exchange with calcium carbonate exoskeleton of coral, when implanted in the rectus abdominis of adult rabbits, dogs and baboons [27]. The long term bone ingrowth of HA implants in humans was studied by R. A. Ayers et. al., the results showed porous block HA that supported the metabolic processes of bone in pores over long periods [28]. Furthermore, an *in vivo* study by R. V. Silva et. al., comparing the rate of bone formation to HA and autogenous cancellous bone grafts to repair bone defects in rats. It was found that the defects filled with autogenous calcellous bone grafts and bioceramic materials, hydroxyapatite, showed a similar volume of bone tissue within the defect [29]. Additionally, the osteointegration of bioceramics fragments in this study allowed the reconstruction of parietal bone defects without the need for a bone graft.

(iv) Resorbable materials: The implant chemically dissolves or is removed from the body by cells. There is no fixation, only replacement of implant with biological tissues such as tricalcium phosphate, and biodegradable polymers [11]. The most popular ceramic with high biodegradability is tricalcium phosphate and it has attracted considerable attention among biomaterials. However, some reports on biodegradable porous tricalcium phosphate implanted alone at extraskeletal sites suggest that degradation occurred quickly with no bone formation. Therefore, the implants are made of bioresobable or degradable materials with biocompatibility for eliminating host immune rejection response, biodegradability for ensuring complete tissue regeneration in the repair area and osteoconduction for facilitating bone formation [6]. For example,

C. Balcik et. al [19] suggested that the mixture of HA/TCP (60/40) could be used in the repair of segmental bone defects due to its good bioactivity and higher biodegradability than pure HA.

2.3 Calcium Phosphate Based Bioceramics

Calcium phosphate based bioceramics (mainly hydroxyapatite and tricalcium phosphate) are the largest and most popular group of materials that are widely used as bone replacement materials in the field of orthopedic, dentistry, and surgery and are used as a matrix for controlled drug release in medical application because of their similarity in composition to the mineral phase of natural bone [23,30]. There is a variation of mechanical properties of synthetic calcium phosphate as given in Table 2.4. Calcium phosphate found in nature as a rock mineral in Morocco, Israel, Philipines, Egypt, Russia and in smaller quantities in other countries [14]. The natural form is not completely pure and there are some other components like sand and lime which can change the composition. In terms of P_2O_5, most calcium phosphate rocks have a content of 30 to 40% P_2O_5 in weight [31]. Calcium phosphate exists in different form and phase depending on temperature, partial pressure of water and the concentration of impurity, such as hydroxyapatite (HA), tricalcium phosphate (TCP), tetracalcium phosphate (TTCP) and the other phases. The different phases have a resulted in the properties of calcium phosphate ceramics. Table 2.5 summarizes the physical properties of various forms of calcium phosphate.

Table 2.4 Physical properties of calcium phosphate [10]

Properties	Value
Elastic Modulus (GPa)	40-117
Compressive strength (MPa)	294
Bending strength (MPa)	147
Hardness (Vickers, GPa)	3.43
Poisson 's ratio	0.27
Density (theoretical, g/cm^3)	3.18

Table 2.5 Physical properties of various phases of calcium phosphate bioceramics [32]

Phase	Chemical formulae	Ca/P ratio	Crystal structure	Density (g/cm^3)
Hydroxyapatite (HA)	$Ca_{10}(PO_4)_6(OH)_2$	10/6	Hexagonal, $P6_3/m$ space group, Cell dimensions; a=b=9.42 A^o and c= 6.88 A^o	3.16
α-Tricalcium phosphate (α-TCP)	α-$Ca_3(PO_4)_2$	3/2	Monoclinic, $P2_1/$ a space group, Lattice constants; a=12.887 A^o, b=27.280 A^o, c=15.219 A^o, β=126.20^o	2.86
β-Tricalcium phosphate (β-TCP)	β-$Ca_3(PO_4)_2$	3/2	Pure hexagonal, Rhombohedral, space group R3cH, unit cell dimensions; a=b= 10.439 A^o, c=37.375 A^o, and α=β=90^o, γ=120^o	3.07
Tetracalcium phosphate (TTCP)	$Ca_4P_2O_7$	2/1	Monoclinic, space group $P2_1$, a=7.023, b=11.986, c=9.473; β=90.90^o	3.05

Figure 2.4 and Table 2.6 show the amount dissolved at equilibrium depends on the thermodynamic solubility product of the compound and the pH of the solution. The relative solubility of hydroxyapatite compound at pH above 4.2 is shown. Below this, dicalcium phosphate dehydrate ($CaHPO_4.2H_2O$) is the stable compound at a given pH. Under normal conditions of pH 7.2, hydroxyapatite is the stable calcium phosphate compound. This may drop to as low as pH 5.5 in the region of tissue damage, although this would eventually return to pH 7.2 over a period of time. Even under these conditions, hydroxyapatite is still the stable phase. At pH 7.0, the solubility decreased in the order of TTCP> α-TCP> DCPD> DCPA> β-TCP> HA. Therefore, HA is the stable phase at that pH and is the ideal phase for any applications inside the human body

because of its excellent stability above pH.4.2, human blood pH being 7.3. Table 2.7 shows the main calcium phosphate compounds that have a biological use as surgical materials. Different phases of calcium phosphate ceramics are used by the modern health care industry, depending upon whether a resorbable or bioactive material is desired.

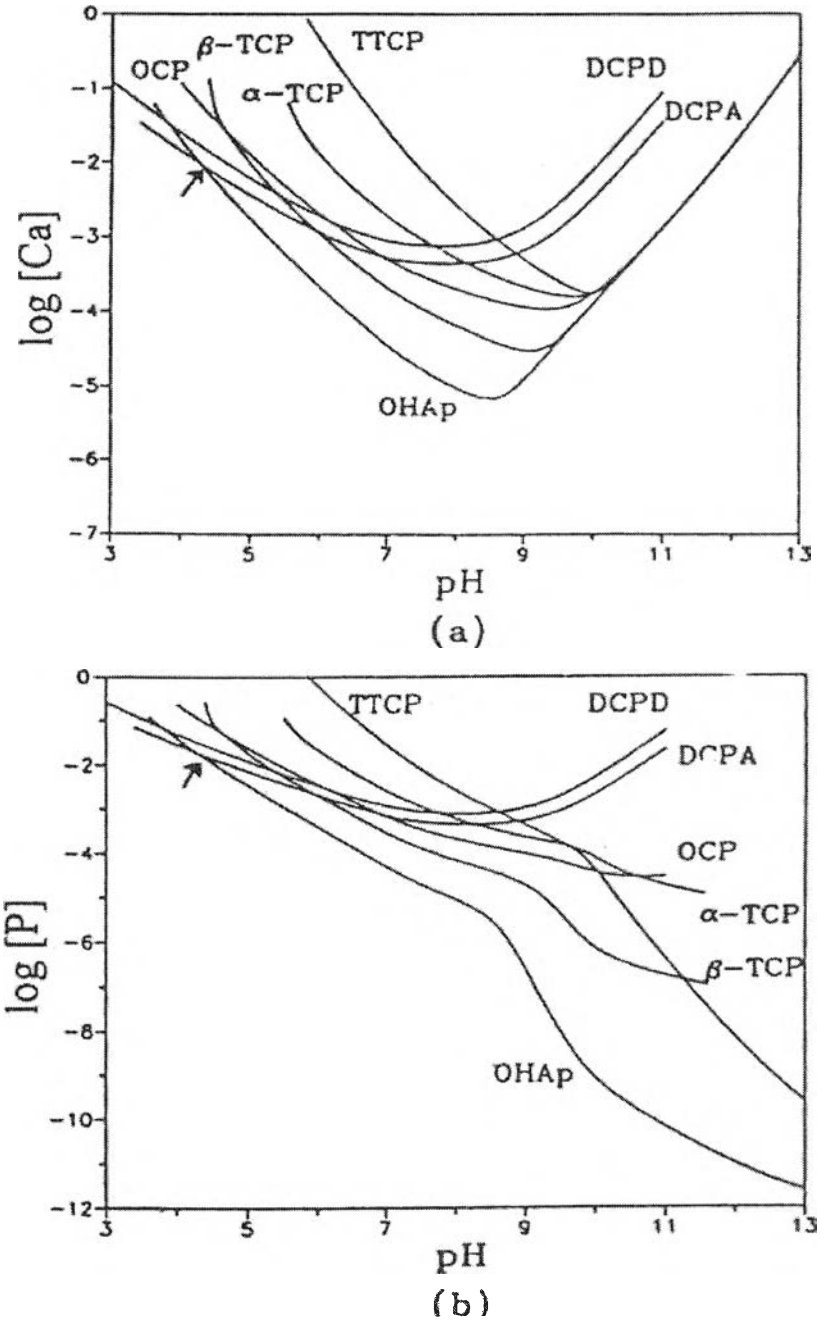

Figure 2.4 Solubility phase diagrams for the ternary system,$Ca(OH)_2$-H_3PO_4-H_2O at 37 °C showing logarithmic graphs of the concentrations of (a) calcium phosphate (b) phosphate as a function of the pH in solution saturated with various salt in the solubility isotherm [1].

Table 2.6 Solubility and pH stability of different phases of calcium phosphates [32]

Phases	Solubility at 25 °C, -log (K_{sp})	pH stability range in aqueous solution at 25 °C
Hydroxyapatite (HA)	116.8	9.5-12
β–Tricalcium phosphate (β-TCP)	28.9	Cannot be precipitated from aqueous solution
α–Tricalcium phosphate (α-TCP)	25.5	Cannot be precipitated from aqueous solution
Tetracalcium phosphate (TTCP)	38-44	Cannot be precipitated from aqueous solution
Dicalcium phosphate dehydrate (DCPD)	6.59	2.0-6.0
Dicalcium phosphate anhydrous (DCPA)	6.90	Stable at temperature above 100 °C
Amorphous calcium phosphate (ACP)	Cannot be measured precisely. However, the following values were reported: 25.7 ± 0.1 (pH 7.40), 29.9 ± 0.1 (pH 6.00), 32.7 ± 0.1 (pH 5.28)	Always metastable. The composition of a precipitate depends on the solution pH value and composition.
Calcium-deficient hydroxyapatite (CDHA)	~ 85.1	6.5-9.5

Table 2.7 Calcium phosphate compounds used a biomaterial [11]

Name and formula	Abbreviation	Type of compound	Occurrence
Stoichiometric HA $Ca_{10}(PO_4)_6(OH)_2$	S-HA	Ceramics Plasma sprayed coating Coating Composites Drug carrier	close to enamel tissue
Non-stoichiometric apatite*	NS-HA	Low temperature coating Composites Drug carrier	Bone tissue ectopic calcification end term of Ca-P cement
β-or α-tricalcium phosphate $Ca_3(PO_4)_2$	β-or α- TCP	Ceramics composites cements Plasma sprayed coating Drug carrier	High temperature phase impurities in plasma sprayed coating
Dicalcium phosphate dehydrate $CaHPO_4.2H_2O$	DCPD	Cements	Forms also during Ca-P cement setting
Anhydrous phosphate dehydrate $CaHPO_4$	ADCP	Cements	
Octacalcium phosphate $Ca_8(PO_4)_4(HPO_4)_2.5H_2O$	OCP	Cements	Forms also during Ca-P cement setting
Tetracalcium phosphate $Ca_4(PO_4)_2.O$	TTCP	Cements	Impurity in plasma sprayed coating
Amorphous calcium phosphate*	ACP	Cements Drug carrier Low temperature coating	Biomineralizations plasma sprayed coating

* Several chemical formulae have been proposed

Hydroxyapatite ($Ca_{10}(PO_4)_6.H_2O$) is a calcium phosphate based ceramic and it is the name given to the apatite family of minerals as follows:

$X_{10}B_4A_2$, Where X could be replaced by monovalent ions, Na^+, K^+, Li^+, divalent ions, Sr^{2+}, Ba^{2+}, Pb^{2+}, Mg^{2+}, Mn^{2+},Sn^{2+}, Zn^{2+} and polyvalent Al^{3+}

A could be replaced by anionic substitute (OH^-, F^-, Cl^-, $1/2CO_3^{2-}$)

B could be replaced by the PO_4^{3-}, CO_3^{2-}, HPO_4^{2-}, SO_4^{2-}, MnO_4^-, VO_4^{2-} and BO_3^{3-} group

Hydroxyapatite crystallizes in the hexagonal crystal system with a $P6_3/m$ space group and cell dimensions of a = b = 9.42 A^o, and c = 6.88 A^o, where $P6_3/m$ refers to a space group with a six –fold symmetry axis with a three-fold helix. The microstructure of HA is given in Figure 2.5 [10].

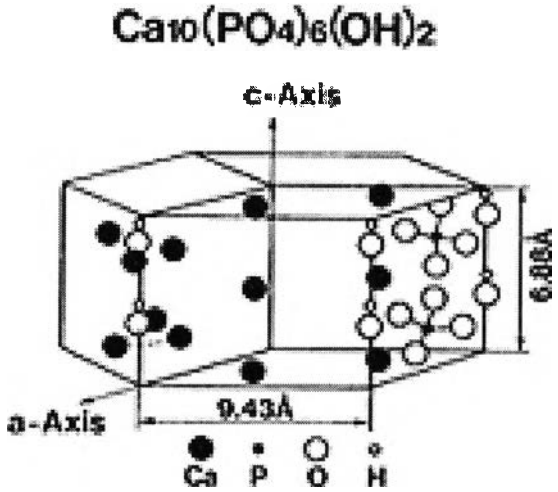

Figure 2.5 Structure of hydroxyapatite.

The atomic structure of hyroxyapatite projected down the c axis onto the basal plane is shown in Figure 2.6. The hydroxyl ions lie on the connected basal plane and they occur at equidistant intervals one-half of the cell (3.44 A^o) along columns perpendicular to the basal plane and parallel to the c axis. Six of the ten calcium ions in the unit cell are associated with the hydroxyls in these columns, resulting in strong interactions among them [10]. HA has a specific gravity of 3.16 g/cm^3 and is 5 on the

Mohrs hardness scale. Pure hydroxyapatite powder is white color [31]. Hydroxyapatite is the ideal phase for the applications inside human body because it has the capacity to form a chemical bond with bone tissue at the interface and can induce a continuous transition from bone tissue to implant surface. HA is one of the materials that are classed as bioactive materials, meaning that it will support bone ingrowth and osteointegration when is used in orthopedic, dental and maxillofacial applications. The schematic of the bone like apatite formation on the HA surface in SBF is shown in Figure 2.7 [33,34].

Hydroxyapatite can be decomposed to tricalcium phosphate (β and α-TCP) ceramic by firing at temperatures higher than 800 up to 1200 °C according to the stoichiometry of the structure [25,35,36] In general, thermal decomposition of HA at temperatures above 1200 °C will result in the initial formation of oxyhydroxyapatite [$Ca_{10}(PO_4)_6(OH)_{2(1-x)}$], thereby leading to the formation of tricalcium phosphate [$Ca_3(PO_4)_2$] and calcium oxide [CaO] and further heat treatment well result in forming tetracalcium phosphate [$Ca_4(PO_4)_2O$]. This transformation process is similar to previously reported work which has described the natural decomposition of bone [2]. Moreover, thermal stability of either HA or TCP could also be influenced by the incorporated ions in their structure [36-38]. Tricalcium phosphate (mainly β-TCP) is one of the most used bone substitute materials due to its good biocompatibility and bonding directly to bone without any intermediary layer of connective tissue, and its chemical stability and resorbability *in vivo*. Pure TCP ceramics are dissolved and resorbed relatively quickly and do not have good mechanical stability, whereas pure stoichiometrical HA is the least degradable. Biphasic ceramics, mixtures of HA and TCP, have been then considered as potential substitutes for bone replacement in surgical operations for the past two decades owing to their favorable features such as;

(i) difference in dissolution properties, HA is non-resorbable and TCP is resorbable,

(ii) rapid bone formation around the implant site

(iii) its closed matches with the inorganic component of living bone.

For example, C. Balcik et. al. demonstrated the comparison of pure HA and HA/TCP (60/40) ceramics in repair of segmental bone defect of rabbits. It was found that the radiological grade of healing and bonding to bone were slightly better in HA/TCP (60/40) composite ceramics than pure HA ceramics, which is possibility due to the faster degradation of TCP [19]. However, from a mechanical point of view, both HA and TCP present low mechanical properties comparing to bone[11]. Consequently, one of the main drawbacks of biphasic ceramics is their brittleness and their low mechanical strength, which limits their use in case of important stresses. Therefore, it is necessary to optimize the mechanical properties of calcium phosphate bone substitutes.

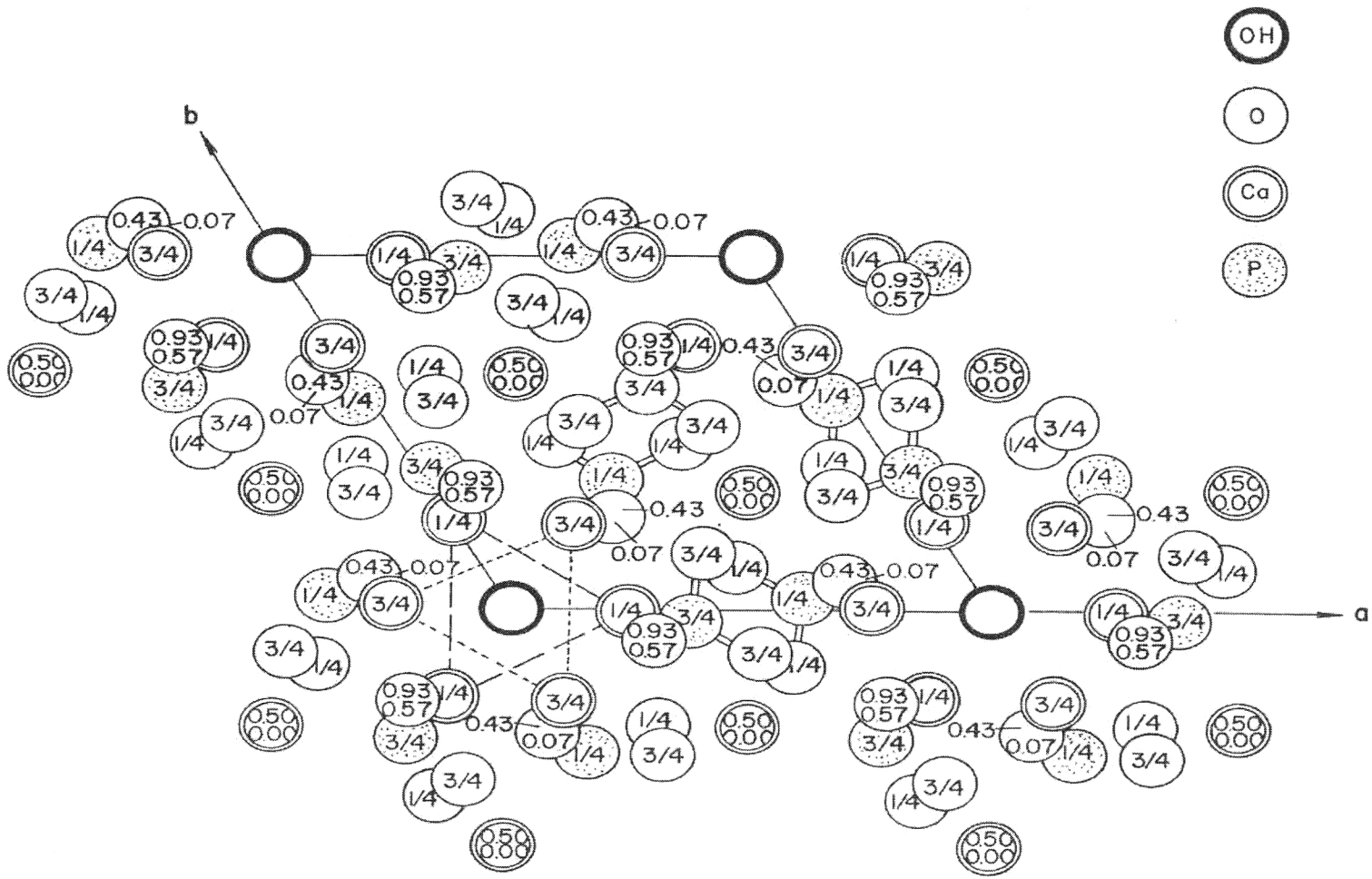

Figure 2.6 Hydroxyapatite structure projected down the *c* axis onto the basal plane.

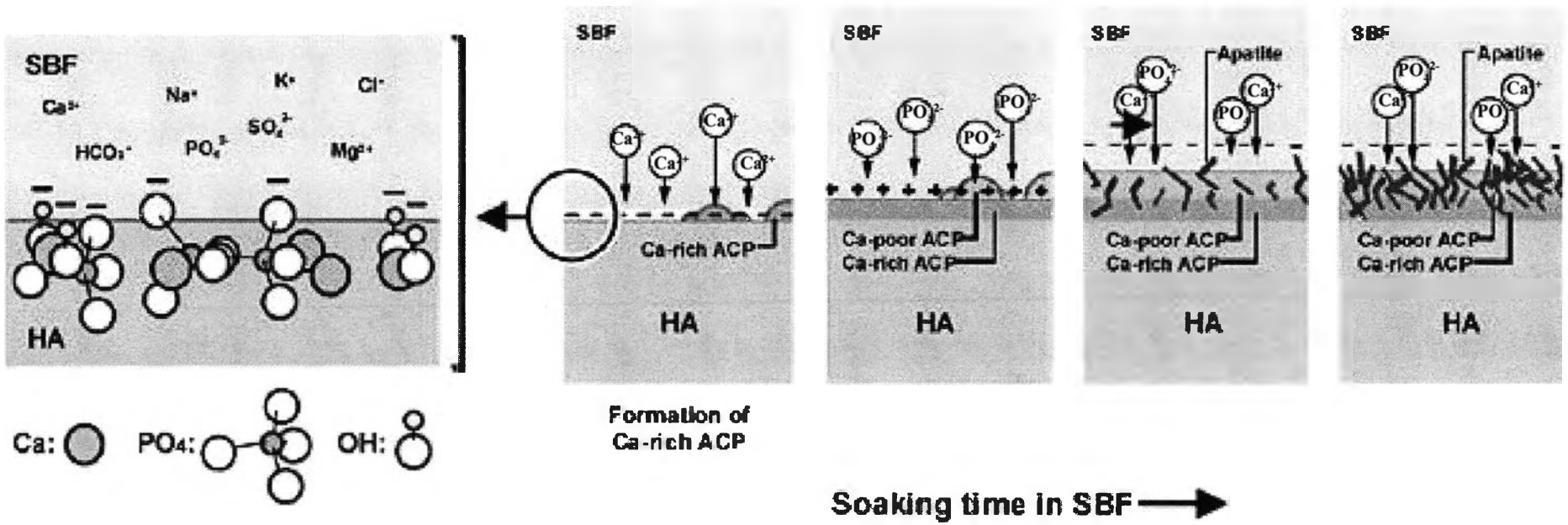

Figure 2.7 Schematic explanation of the negative charge occuring on the HA surface and the process of bonelike apatite formation immersion in SBF.

2.4 Preparation Methods of Porous Hydroxyapatite for Bone Replacement Applications

Although hydroxyapatite bone replacement shows very good bone regeneration for large bone defects or for such situations that the bone healing process is difficult, bone grafts are required. Therefore, the continued development of hydroxyapatite based porous bone replacement is of major interest. The pore structure of HA implant is regarded as a main factor in promoting osteoinduction with the intention that implanted porous ceramic will be progressively replaced by natural bone due to its porous structure allowing cell attachment, proliferation, and to provide pathways for biofluids. Additionally, porous HA exhibits strong bonding to bone due to the pores which provide a mechanical interlock leading to a firm fixation of the material. Bone tissue grows well into the pores, increasing strength of the HA implant [2,19,38].

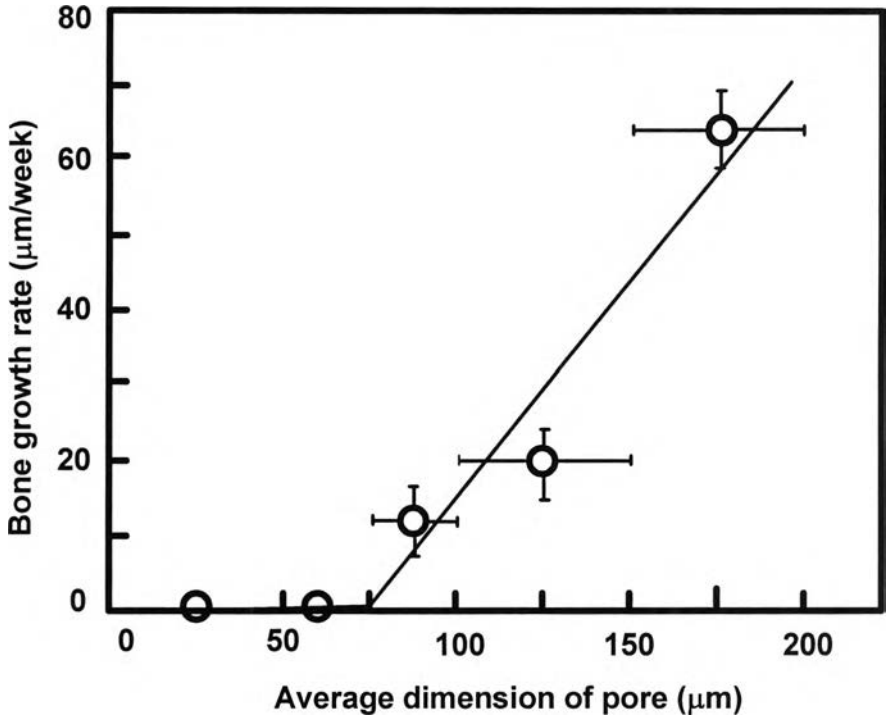

Figure 2.8 Variation of the bone growth rate as a function of the pore dimension under constant porosity conditions [11].

The variation of the bone growth rate as a function of the pore dimension under constant porosity conditions is shown in Figure 2.8. From this graph it is possible to infer that pores with diameter significantly less than 100 μm do not permit cell and tissue colonization [11]. It has been reported that the minimum pore size required for bony ingrowth of the surrounding bone together with blood supply is about 100-200 μm for macropores or even as large as 250-400 μm [11,19,22,38]. Moreover, pores sizes between 100 and 1000 μm play an important role in cellular and bone ingrowth, being necessary for blood flow distribution and having a predominant function in the mechanical strength of the substrate. Finally, the presence of pores size greater than 1000 μm will have an important role in the implant functionality and in its shape [34]. The sufficient pore sizes allow the growth of osteons of vascular connective tissue, which provides a lattice and cellular environment ability to form appositional bone by a process that resembles the process whereby cancellous bone is converted to compact bone by successive deposition of lamellae on to the new bone a process which originates a series of primary osteons. Therefore, designed porous scaffolds should have networks of interconnected pores where more than 60% of pores should have a size greater than 50 μm.

M. Fabbri et.al (1995) studied the properties and preparation of porous HA aggregates for medical applications [12]. Afterwards, the effect of pore size *in vivo* behavior of ceramic scaffold has been studied by D.M. Arm et.al (1996). They studied the effected of *in vivo* bioactivity of porous coralline HA on animals. It was found that the microporous structure is similar to bone, containing interconnecting pores of 190-230 μm in diameter, allowing for vascular ingrowth and bone deposition. Pore diameter which is $\leq$200 μm is considered to be the most suitable size for ingrowth of vessel sprouts and woven bone [39]. Moreover, many researchers studied this field, such as R. A. Ayers et.al (1998) who reported on the ingrowth of bone into coralline, porous HA block in orthopedic surgery. Z. Zyman et al (1998), presented results on the formation of bone tissue in pores and around a block made of partially dehydroxylated porous HA ceramic, with pore sizes of 140-200 μm, and implanted into a rat's femur [28,35].

Therefore, the most important properties with respect to the use of hydroxyapatite as a biomaterial for bone implant are dependent on the pore size, high porosity and the interconnectivity pore system of the materials due to their influence on the implant's final behavior [22]. Some of the typical methods of fabricating porous hydroxyapatite ceramics are described below:

2.4.1 Conversion of Marine Coral and Natural Bone Skeleton by Hydrothermal Processes

Some ceramics are naturally derived and have a distinct porous structure of their own such as coral, animal or human bone [3,27,40]. The three dimensional skeletal structure of certain marine corals and human cancellous bone can be used as a template for making porous structures. The coralline HA from the hydrothermal exchange technique contains architecture that replicates the exoskeletal structure of the corals [27]. The process used to make the coralline HA does not change the existing internal architecture of the coral. The resulting porous HA for this method has many sizes of macropores of diameter ~ 200-500 μm with good interconnectivity and 50-65% porosity, and these are enough for its usefulness in obtaining artificial bone substitutes [4,39]. B. Ben-Nissan et.al (2004) studied the effected of HA coating on natural coral by sol-gel method and found that the pore size of coral investigated in the new species is between 150 and 300 μm with interconnections of about 100-150 μm. [28]. A drawback of these materials is the difficulty in controlling porosity. Mechanical strength that can effect to the materials cannot be tailored for a specific purpose and the limited amount of the marine coral is another obstacle [3,4].

2.4.2 Formation of Porous Structure using Pore Forming Volatile Particles

Various kinds of pore making agents including paraffin, naphthalene, carbon, starch, flour, hydrogen peroxide, or polymers such as polyvinyl butyral which burn away during sintering are admixed to HA powders or slurries [5,20,41]. This approach allows direct control over the pore characteristics since their fraction, size, morphology, and distribution are controlled by type, amount and properties of the added volatile phase. The porous ceramics usually have closed macropores with a varied pore size of 0.1-5000 μm diameter [9]. An example of a manufacturing process of macropous ceramics are shown in Figure 2.9,which uses slip casting for shaping and polymethylmethacrylate (PMMA) as pore generating agent which allows significant control of the porous β-TCP structure. Figure 2.10 shows PMMA balls as a pore generating agent and the porous structures obtained after debinding and sintering treatments [42]. Moreover, Dean-Mo Liu [43] studied the preparation of porous HA bioceramic via a slip casting route and the foaming agent used was polyvinyl butyral (PVB). It was found that pore size in this method was approximately 160-200 μm, closely resembling that of the starting PVB particle size, ~180 μm on average. After that, he fabricated the porous hydroxyapatite granules using polyvinyl butyral (PVB) as a forming agent. This method could fabricate porous HA granules which were successfully and easily made using a dip-casting technique. The granules could be obtained with a wide range of porosity from 24 to 76%, pore size from ~95 to ~400 μm, and granular sizes from 0.7 mm to ~4 mm [21,42]. The organic particle embedding technique can be controlled over a range of size and shapes for porous structure ceramics. On the other hand, they do not provide any control over the connection patterns and often leave blind ends to the implants.

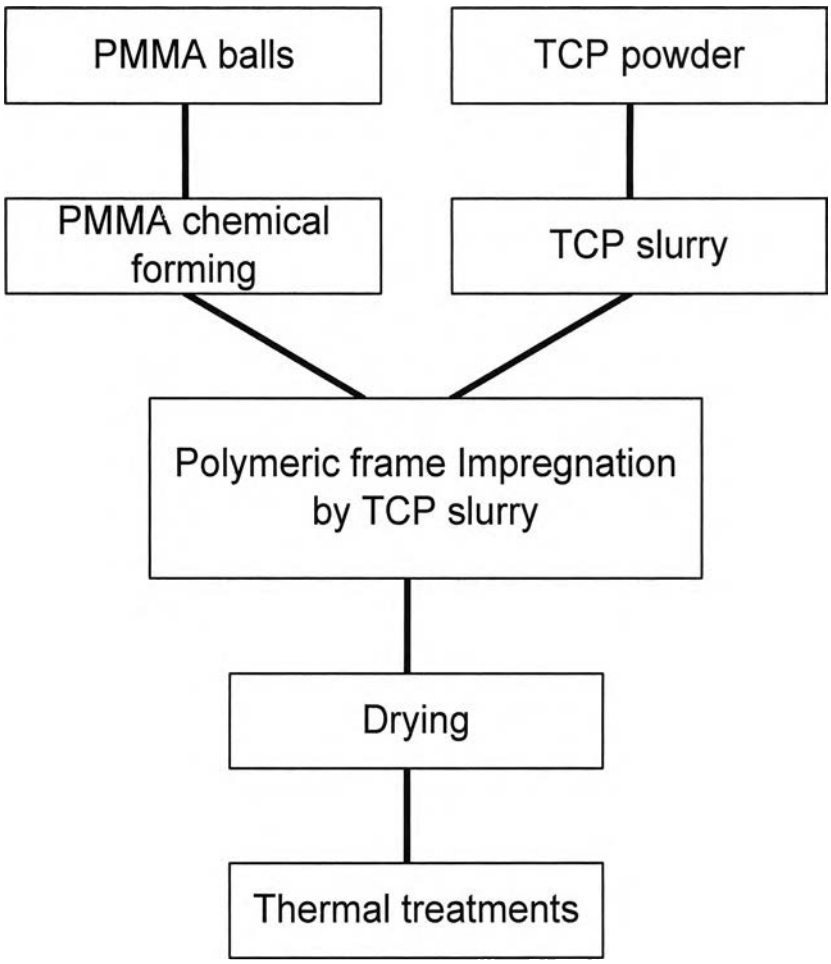

Figure 2.9 An example of a manufacture process of macroporous body [44].

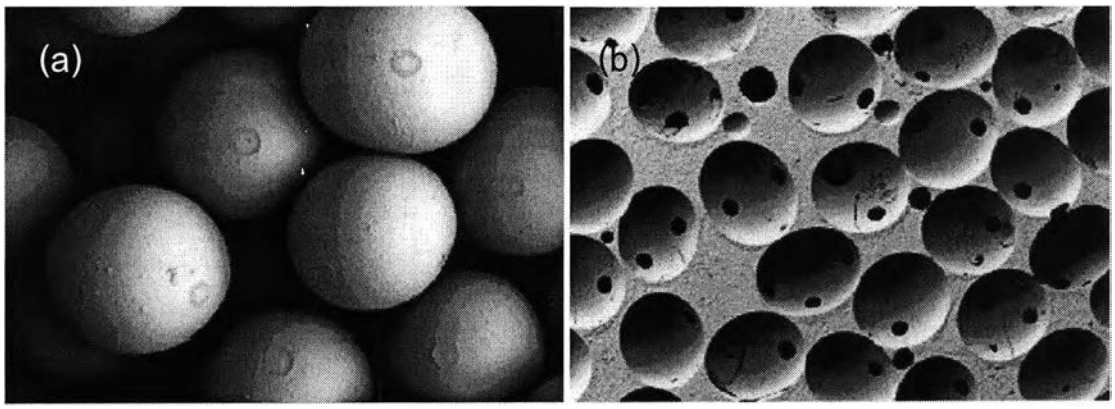

Figure 2.10 (a) PMMA balls as pore generating agent (diameter balls: 190 μm) and (b) porous structures obtained after debinding and sintering treatments (pore diameter: 110 μm) [44].

2.4.3 Ceramic Foaming Technique

This technique involves foaming ceramic suspensions of ceramic green bodies via gas evaporating chemical reactions from organic and inorganic sources. Some foaming agents tested were hydrogen peroxide, carbonate salt, and baking powder [20,27,42,45]. They were added to the HA slurries, stirred to unit formation of foam occurred and then subjected to polymerization, followed by sintering. Porous HA obtained has pore sizes of 30-600 μm [25,42]. The disadvantage of this technique is that it typically results in a structure of poorly interconnected pores and none of an uniform pore size distribution.

A. Almirall et.al (2004), prepared the α-TCP scaffolds in cement using hydrogen peroxide (H_2O_2) as foaming agent. It was found that these scaffolds combine an interconnected macroporosity with a high microporosity and can be obtained at low temperature. The percentage of porosity, and the pore size and shape can be controlled by different content of foaming agent [42].

L.A. Cyster et. al (2005) fabricated porous HA using methylcellulose as the foaming agent. It was found that methylcellulose could induce pores in the structure. The HA porous ceramics produced presented a highly interconnected porous network with average pore size ranging from 391 ± 39 μm to 495 ± 25 μm, the window size ranging from 73 ± 5 μm to 135 ± 7 μm. Pore diameters obtained were controllable in the range 200-500 μm [45].

2.4.4 Polymeric Sponge Method

The polymeric sponge method is a common method for the production of porous hydroxyapatite. This method is performed by impregnating porous polymer sponges with slurries [5]. Figure 2.11 shows step- by –step details of polymeric sponge method. The polymer, having the desired pore structure, simply serves as a sacrificial template for the ceramic coating. The polymer template, from which the ceramic or glass scaffold copies the shape, can be produced in an irregular or complex shape that can match the bone defect shape and size of an individual patient [21]. Moreover, this method does not involve the use of any toxic chemicals and is suitable for commercialization in terms of simplicity and low cost. Porous HA prepared via the polymeric sponge method has shown well-interconnected pores but poor mechanical strength for load bearing applications. It was shown that the polymeric sponge method results in a proper pore size distribution as osteoconduction requires. This is characterized by the existence of micro-meso-macropores with an adequate degree of interconnection.

H. R. Ramay et.al (2003) prepared porous HA scaffolds by combination of the gel-casting and polymer sponge methods. The scaffolds in these results had a pore size ranging 200-400 mm and high interconnectivity. Because the macroporous structure of the scaffold product was the replicate of the polymer sponge template, the pore size and shape were controlled and scaffolds of complex shapes could be fabricated [46].

A. C. Queiroz et.al (2004) presented the porous HA for controlled drug delivery by immersing a polyurethane foam sponge in a slurry. From this technique, it could be inferred that the porosity of the samples produced was 73% and with a pore size of 150-400 μm. The morphology obtained appeared to be well adapted to bone growth and drug adsorption [24].

X. Miao et. al (2004) reported that the preparation of porous calcium phosphate ceramics by coating polyurethane foams with calcium phosphate cement. The method resulted in high porosities (~70%), highly interconnected pores and it had macropore sizes of about 1 mm and micropore sizes of about 5 μm [47].

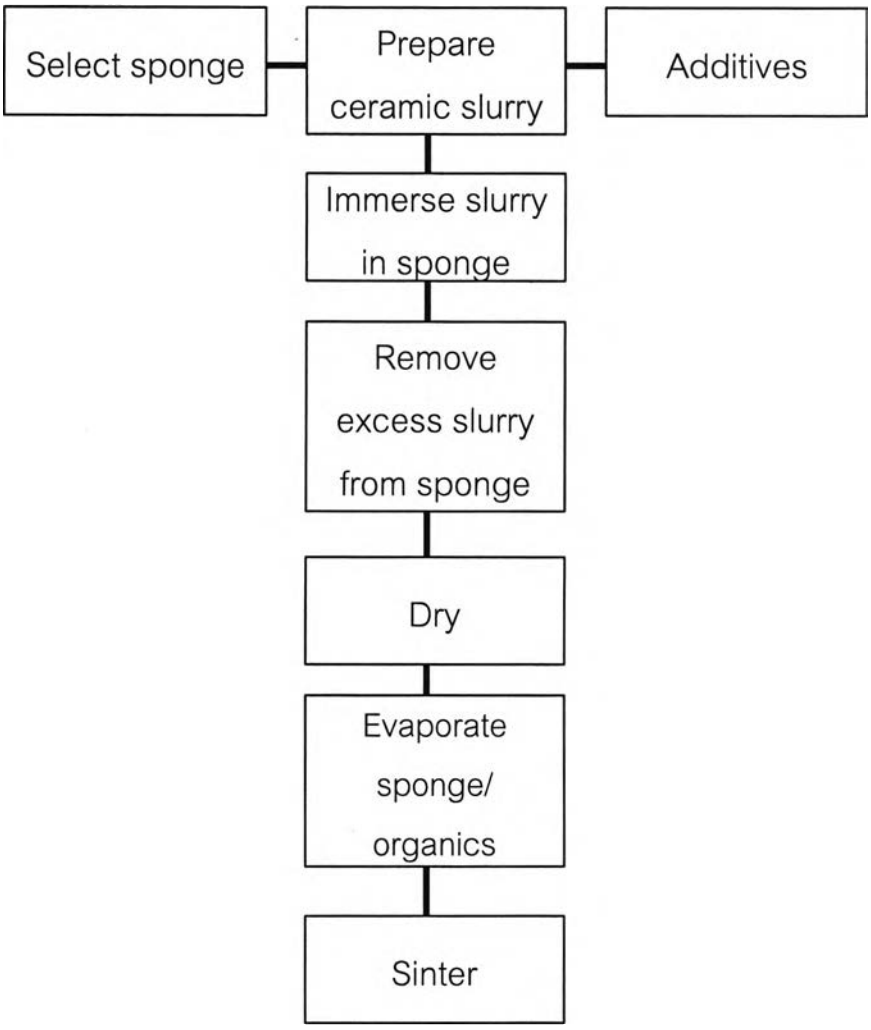

Figure 2.11 Step- by –step details of a polymeric sponge method [5].

2.5 Development of Porous Hydroxyapatite for Bone replacement by polymeric sponge method

Porous HA has been applied for cell loading as a drug releasing agent and has been used extensively for hard tissue scaffolds [22,24,39,48]. Mammalian cells can be grown and maintained *in vitro* but are generally anchorate dependent, i.e. they need a solid substrate for growth [19,27,29,49]. Therefore, porous hydroxyapatite which has an open pore structure is of particular interest and importance due to its porous structure having a large surface area, as well as excellent biocompatibility for use as a bone implant material. However, it is well known that the mechanical strength of a material generally decreases as its porosity increase, but high porosity improved the biological properties of materials [21,45,49,50]. The porous HA ceramics formed by the polymer sponge method have poor mechanical strength for load-bearing applications. Therefore, several researchers have attempted to solve these problems by improving fabrication techniques which allowed the production of biologically and mechanically stronger ceramics that can withstand loading conditions of weight-bearing bones.

2.5.1 Improved Mechanical Strength

Compressive strength of porous human bones varies between 2 and 12 MPa for cancellous bone and between 100 to 230 MPa for cortical bone [37,46]. As prepared artificial porous HA has mechanical strength is rang of 1.3-16 MPa, but bone ingrowth leads to the enhanced compressive strength of porous implants [21,29]. Therefore, many reinforcements, including metallic particles, ceramic whickers, nanoparticles, as well as inert ceramic phases such as Al_2O_3, SiO_2, ZrO_2, have been used in HA materials [9,49,50].

H. R. Ramay and M. Zhang (2003) used a technique that combined a gel-casting method with a polymer sponge method to prepare macroporous hydroxyapatite scaffolds. This novel technique resulted in porous HA with improved mechanical

strength and controlled pore structure. It was found to have a homogeneous microstructure and an open, uniform and interconnected porous structure with a pore size of 200-400 μm. The compressive yield strength of ~5 MPa equivalent to that of cancellous bone and a compressive modulus of ~8 MPa similar to that of cortical bone were achieved [46].

B. Ben-Nissan et.al. (2004) studied the effected of HA coating on natural coral by sol-gel method. This method resulted in improved mechanical strength and interconnection of pores. The pore size of coral investigated in the new species was between 150 and 300 μm with interconnections of about 100-150 μm. HA coated coral had a biaxial strength which was higher than the converted coral and pure coral, a biaxial strength of 13.3± 6.5 MPa for converted and HA coated coral, 7.6 ±1.4 MPa and 6.5 ± 2.9 MPa for coral, respectively [4].

K. Yoshida et.al. (2006) reported the fabricated of hydroxyapatite doped with 3 mol% of Y_2O_3 partially stabilized ZrO_2 by an extrusion process. In this process, the bulk density and bending strength of composites increased with increasing temperature (1300 °C and 1350 °C) and the values ranged from 3.4 g/cm^3 to 3.8 g/cm^3 and 70 MPa to 100 MPa, respectively [16].

X. Wang et.al (2006) improved mechanical strength of porous biphasic phase ceramics, HA/tricalcium phosphate, via microwave sintering. Through the process optimization, such as the sintering, heating rate and the holding time, porous biphasic bioceramics with average crystal size of 300 nm, porosity of 65%, and compressive strength of 6.40 MPa were prepared, giving cancellous bone having a compressive strength of 2-12 MPa [25].

X. Miao et. al. (2007) improved mechanical properties, compressive modulus and maintained the desirable bioactivity of porous HA by coating calcium phosphate cement on polyurethane foam with an open micropore of the struts which were infiltrated

with poly(lactic-co-glycolic acid; PLGA) to achieve an interpenetrating bioactive ceramic/biodegradable composite. In this method, The PLGA filled struts were further coated with a 58S bioactive glass (33wt%)-PLGA composite coating. The PLGA bioactive glass modified porous calcium phosphate ceramic proved to be bioactive and exhibited compressive strength up to 7.7 MPa and compressive module up to 3 GPa, which were comparable to those of natural sponge bones [51].

Moreover, coatings of hydroxyapatite is often applied to metallic implants (most commonly titanium, titanium alloys and stainless steel), oxide ceramics having high strength, and other fibers to alter the surface properties, and to support high mechanical loads, and to meet the biological requirements [15-18]. In this manner, the body sees hydroxyapatite material which it is happy to accept. Therefore, after implantation these materials are covered by fibrous tissue which isolates them from the surrounding living bone and may allow bonding to the living bone, which gives very high effective bone regeneration and accelerates the growth of bone tissue.

2.5.2 Biological Improvement of Porous HA using Ion Substitution

Many samples demonstrate excellent mechanical properties but the materials are bioinert. Thus the bioactivity of HA is one of its advantages as a bioceramic, which could possibly decrease with increasing amounts of additive materials [6,9]. The synthesis of chemically modified or ion-substituted HA has drawn great interest, since the ions played an important role in developing artificial bone with enhanced mechanical properties and biological properties which were the most important features of an implant [40]. Trace ions such as Si^{2+}, Na^{+}, Mg^{2+}, F^{-}, Fe^{3+}, CO_3^{2-} substituted in the HA structure can be have an effect on the lattice parameters, crystallinity, dissolution kinetics and other physical properties of HA implants [38,52,53,54]. Several comparative studied have also simultaneously demonstrated the efficiency of bioactive glass as a better biodegradable ceramic than HA where the degradation of bioactive glass promotes bone apposition and enhances a relatively greater proportion of bone

formation and bonding in vitro [6,38,55,56]. Additionally, silica plays an important role in the biomineralization of many organisms due to the high density of surface silanol groups (Si-OH). The silanol groups tend to be more effective at inducing HA formation in SBF solution [40,55,56]. Therefore, silica and silicate glasses have been widely used as a surface modification agent for tailoring the surface and interfacial properties of the powder [6,57,58]. As example of calcium phosphate apatite formation on the $CaO\text{-}SiO_2$ glasses in SBF is shown in Figure 2.12.

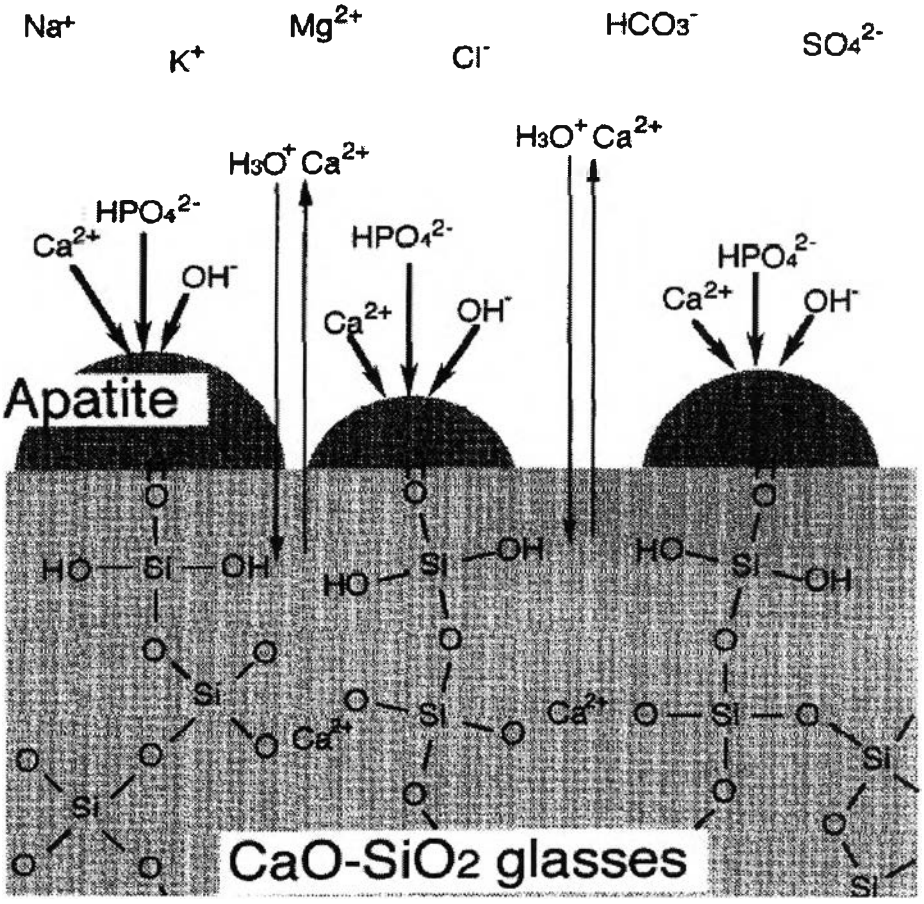

Figure 2.12 Mechanism of apatite formation on $CaO\text{-}SiO_2$ glasses in SBF [56].

A.E Porter et.al (2003) suggested that the incorporation of silicate ions into HA leaded to an increased rate of dissolution of Si-HA. The Ca, P and Si ions subsequently diffused through the ceramic grains to the bone/HA interface, driven by a concentration gradient. The increased concentration of Ca, P and Si ions at the HA ceramic interface

accelerated the precipitation of biological apatite and induced bone replacement at the surface of the ceramic [7].

Y. H. Kim et.al (2005) studied the effect of silicon on the mechanical strength and bioactivity on porous HA ceramics. Porous silicon-incorporated hydroxyapatite was been prepared using natural coral as a calcium source and to obtain a biomaterial having a good biocompatibility. The Si-incorporated hydroxyapatite was prepared by repeating hydrothermal and solvo-thermal processes of the natural coral. It was found that the compressive strength of the porous silicon-incorporated hydroxyapatite at 70% porosity was 5.5 MPa and silicon content ranged from 0.12% to 0.19% by weight [37].

K. So et al. (2006) confirmed the improvement of bone bonding ability by adding glass composite (CaO:MgO:SiO_2 based glass) to HA. They suggested that the glass containing HA showed higher degradability and compressive strength than the controlled HA [8].

M. F. Morks (2008) studied the effective of fused silica mixed with HA powder on stainless steel by plasma spraying. He found that the sprayed HA coating with 10 and 20wt% SiO_2 had a dense structure with low porosity compared to the pure HA coating. The presence of silica significantly improved the adhesive strength, hardness, and abrasive wear of HA/SiO_2 coating surface, mainly due to the increase in bonding strength of the coating at the interface [55].