SCHOOL OF MATERIALS AND MINERAL RESOURCES ENGINEERING

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FABRICATION OF POROUS β-TRICALCIUM PHOSPHATE GRANULES CEMENTS BY USING β-TRICALCIUM PHOSPHATE GRANULES AS STARTING MATERIALS

By

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DECLARATION

I hereby declare that I have conducted, completed the research work and written the dissertation entitled "**Fabrication of Porous β-Tricalcium Phosphate Granules Cement by Using β-tricalcium Phosphate Granules as Starting Materials**". 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.

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Finally, I would like to dedicate this thesis to my parents, En. Abd Halim Hafiz bin Abdul and Puan Normah binti Yew Ahmad. Their sacrifice, love and support meant so much to me which in the end gave me the motivation to complete my studies and research in Universiti Sains Malaysia.

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LIST OF SYMBOLS

β	Beta
α	Alpha
θ	Theta
μ	Micron
◦	Degree
rpm	rotation per minute

LIST OF ABBREVIATIONS

AP	Apparent porosity
α-TCP	α-tricalcium phosphate
BD	Bulk density
β-TCP	β-tricalcium phosphate
β-TCPGC	β-tricalcium phosphate granular cement
DCPD	Dicalcium phosphate dihydrate
FESEM	Field Emission Scanning Electron Microscopy
HAp	Hydroxyapatite
ICDD	International Center for Diffraction Data
L/G ratio	Liquid to granules ratio
L/P ratio	Liquid to powder ratio
PSA	Particle size analysis
XRD	X-ray diffraction

FABRIKASI β-TRIKALSIUM FOSFAT GRANUL SIMEN BERLIANG DENGAN MENGGUNAKAN β-TRIKALSIUM FOSFAT GRANUL SEBAGAI BAHAN PERMULAAN

ABSTRAK

Tujuan kajian ini adalah untuk menghasilkan β-trikalsium fosfat granul simen berliang dengan menggunakan saiz granul yang berbeza iaitu 300-600 μm dan 600-1000 μm. Bagi mencapai tujuan ini, β-trikalsium fosfat granul telah didedahkan kepada 1.0 mol/L larutan asid fosforik dengan nisbah larutan terhadap granul 1:1. Masa bagi spesimen untuk mengeras telah ditetapkan pada 1, 3, 5, 7, dan 24 jam. Spesimen yang telah mengeras akan dianalisa menggunakan XRD bagi mengesan pembentukan fasa dikalsium fosfat dihidrat di dalam spesimen yang telah mengeras. Didapati bahawa semakin kecil saiz β-trikalsium fosfat yang digunakan, semakin tinggi dikalsium fosfat dihidrat yang terhasil. Hasil adalah konsisten dengan keputusan XRD dimana nisbah luas puncak bagi dikalsium fosfat dihidrat untuk saiz β-trikalsium fosfat yang lebih kecil adalah lebih tinggi berbanding dengan saiz β-trikalsium fosfat yang lebih besar. Keputusan SEM menunjukkan banyak kristal dikalsium fosfat dihidrat berhubung silang diantara satu sama lain bagi saiz β-trikalsium fosfat yang lebih kecil. Dengan masa penetapan 24 jam menggunakan 300-600 μm saiz granul peratusan keliangan adalah sebanyak 42.15% manakala saiz granul 600-1000 μm mempunyai 48.76% peratusan keliangan. Dengan peratusan keliangan yang rendah, kekuatan mekanikal menunjukkan kekuatan mampatan tertinggi iaitu 1.16 MPa berbanding dengan peratusan keliangan yang lebih tinggi, menunjukkan hanya 1.00 Mpa. Justeru itu, ini menunjukkan bahawa β-trikalsium fosfat granul yang bersaiz kecil adalah sesuai bagi penghasilan β-trikalsium fosfat granul simen berliang.

FABRICATION OF POROUS β-TRICALCIUM PHOSPHATE GRANULES CEMENT WITH β-TRICALCIUM PHOSPHATE GRANULES AS STARTING MATERIALS

ABSTRACT

The purpose of this study is to fabricate porous β-tricalcium phosphate granular cement using different size of β-tricalcium phosphate granules (300-600 μm and 600-1000 μm). To achieve this objective, β-tricalcium phosphate granules were exposed to 1.0 mol/L of phosphoric acid solution with liquid to granular ratio (L/G) of 1:1. Besides, the setting time used in this study were fixed at 1, 3, 5, 7, and 24 hours. The set specimen were characterized by XRD to detect the formation of dicalcium phosphate dihydrate (DCPD) phase in the set specimen. It was found that when smaller size of β-tricalcium phosphate granules were used, more DCPD formation were observed. This consistent within the XRD result which the peak area ratio of DCPD for small granules size is higher than the bigger granules. SEM results demonstrated that more DCPD crystals were interlocked each other in the case of small size of β-tricalcium phosphate granule. The higher amount of interlocked DCPD crystal will increased the compression strength of set specimens. In contrast, the percentage of porosity decrease with increasing the formation of DCPD crystal. With 24 hours of setting time using 300-600 μm of granules the porosity percentage were 42.15 % while with size of 600-1000 μm it shows 48.76 % of porosity. With lowest percentage of porosity the mechanical strength shows highest compressive strength which is 1.16 MPa compared to higher porosity which shows only 1.00 Mpa. Therefore, it shows that the smaller size of β-tricalcium phosphate granules is appropriate to fabricate porous β-tricalcium phosphate granular cements.

CHAPTER 1

INTRODUCTION

1.1 Background Research

Calcium phosphate based biomaterials are now used in a number of different applications throughout the human body, covering all areas of the body skeleton which also include dental implants. Presently, calcium phosphate are available in in the form of particulates, blocks, cements, coatings, customized design for specific applications (Dorozhkin, 2010). In general, biomaterials are intended to react at interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body. The major difference of biomaterials from other classes of materials is their ability to remain in a biological environment without damaging the surroundings and without being damaged in the clinical process (Dorozhkin, 2015).

Bioceramics can have structural functions as joint or tissue replacements and providing temporary structure and frame works those are dissolved and replaced as the body rebuilds the damaged tissues. One of the well-known bioceramics materials is β-TCP. The main driving force behind the use of β-TCP as bone substitute materials is their chemical similarity to the mineral component of bone and teeth (Al-Sanabani et al., 2013). Besides, β-TCP are non-toxic, biocompatible, not recognize as foreign materials in the body and most importantly shows bioactive behavior. Moreover, β-TCP could also be fabricated as a scaffold in order to provide a template for new bone formation (Thrivikraman & Basu, 2014).

Among the existing calcium orthophosphate, only certain compounds are useful for biomedical applications, because those having a Ca/P ratio less than 1 are not suitable for implantations into the body due to their high solubility and acidity (Raynaud et al., 2002). The use of materials with Ca/P less than 1 is not suitable but materials with Ca/P ratio more than 1 is good as bioceramics materials. Hence, β-TCP are suitable to be used as its Ca/P ratio is 1.50.

Researchers also discovered that the calcium orthophosphate can be tailored as self-setting cements, which are bioactive and biodegradable. After mixing, calcium orthophosphate powder with liquid, it will form a paste which set and harden. Since this calcium orthophosphate powders can be set it will be possess excellent molding capabilities, easy manipulation and nearly perfect adaptation to the complex shapes of bone defects, followed by gradual bioresorption and new bone formation. Currently this formulation are widely used as synthetic bone grafts, with several advantages, such as pourability and injectability (Dorozhkin, 2013).

Previous study have reported that interconnected porous cement were fabricated by exposing α-TCP foam granules with saturated acidic calcium phosphate solution (Shariff et al., 2015). However, the setting time found in this study are difficult to control for clinical application. Since chemical formula of α-tricalcium phosphate granules is same with β-TCP, other study has found that the β-TCP is suit to replace α-TCP as starting material for fabrication of interconnected porous calcium phosphate cement (Shariff et al., 2015). However, this study also face similar problem which setting time issue (Fukuda et al., 2017b). Later recent study found that the setting time of β-TCP granular cement could be regulated

by adding certain concentration of citric acid in saturated acidic calcium phosphate solution (Fukuda et al., 2017a).

1.2 Problem Statement

β-TCP shows good osteoconductivity and compatibility after implanted in the bone defect area. Since, the solubility of the β-TCP is lower than the α-TCP, it is possible to prolong the setting time. Another issue occur when cement harden is that pores formation inside the cement are not interconnected and small. Therefore, up to date many studies have reported that the method to produce interconnected porous structure of β-TCP granules cement.

Therefore, recent studies has reported that the usage of β-TCP granules as a starting materials to fabricate porous calcium phosphate cements. By Fukuda et al., (2017) the method use are by mixing the β-TCP granules with saturated acidic calcium phosphate. The result obtained in this study have clearly demonstrated that the β-TCP granules were set when mixed with saturated acidic solution. In other words, dissolution rate of β-TCP granules are enough when mixed with the acidic solution for the setting reaction through the dissolution of β-TCP granules and precipitation of DCPD, and bridging the β-TCP granules with one another with the DCPD crystal. In fact, setting time of the β-TCP granules cement was extremely fast approximately 1 min, which is relatively short for its clinical application even when β-TCP granules are used.

Hence, in latest studies Fukuda et al., (2017) the researcher tried to prolong setting time, by adding retardant in saturated acidic calcium phosphate solution. Retardant used to inhibit growth of DCPD crystal is citric acid. This study succeed to prolong the setting reaction of β-TCP granular cement up to 30 minutes.

By using phosphoric acid as acidic solution, it will then supply phosphate ion to make dissolution precipitation process. While calcium ion solely sourced from β-TCP granules only. From previous study, calcium ion was sourced form acidic solution and β-TCP. Newly, there is no current studies that use phosphoric acid as acidic solution to set β-TCP granules cement. Therefore, in this study we attempted to prolong the setting of β-tricalcium phosphate granules cement by exposing β-tricalcium phosphate granules with diluted phosphoric acid solution.

1.3 Objectives

1. To fabricate porous β-tricalcium phosphate granular cement using different size of β-TCP granules.
2. To investigate the properties of β-TCP granular cement after exposing β-TCP granules with diluted phosphoric acid solution at different setting time.

1.4 Research Approach

In this study the main materials used is β-tricalcium phosphate. β-TCP was obtained by mixing of calcium carbonate ($CaCO_3$) and dicalcium phosphate dihydrate (DCPD) with Ca/P

ratio 1.5. Both raw materials are then mixed by wet mixing process to obtain homogenous mixing using planetary mill. After drying process the mixing powder is then pressed into pellet form. Then, the pellet was sintered using electric furnace. Then sintered pellets were crushed and sieved to get granules according to sizes needed which are 300-600 μm and 600-100 μm. After that, these granules were placed into the mold with 6 mm diameter and 3 mm height. The diluted phosphoric acid solution were dropped into the mold with liquid volume to the granular mass ratio of 1 ml: 1 g at 25 ˚C. There are five different setting times varies in this study which are 1, 3, 5, 7 and 24 hours. After that, the set specimen were washed with acetone to stop setting reaction. Finally, the specimen were dried in oven at 60 ˚C for 1 hour.

To validate the raw materials used in this study particle size analysis (PSA), X-ray diffraction (XRD), field emission scanning electron microscope (FESEM) and energy dispersive X-ray (EDX) were performed to observe particle size, phase composition, morphology and elemental analysis respectively.

The setting β-TCP granules cement were subjected to various characterization tests such as appearance observation, porosity and bulk density, PSA, diametral tensile test (DTS), FESEM, XRD and EDX were used to analyze microstructure, phase and elemental. Figure 1.1 shows the flowchart process of fabrication interconnected porous β-TCP granular cement.

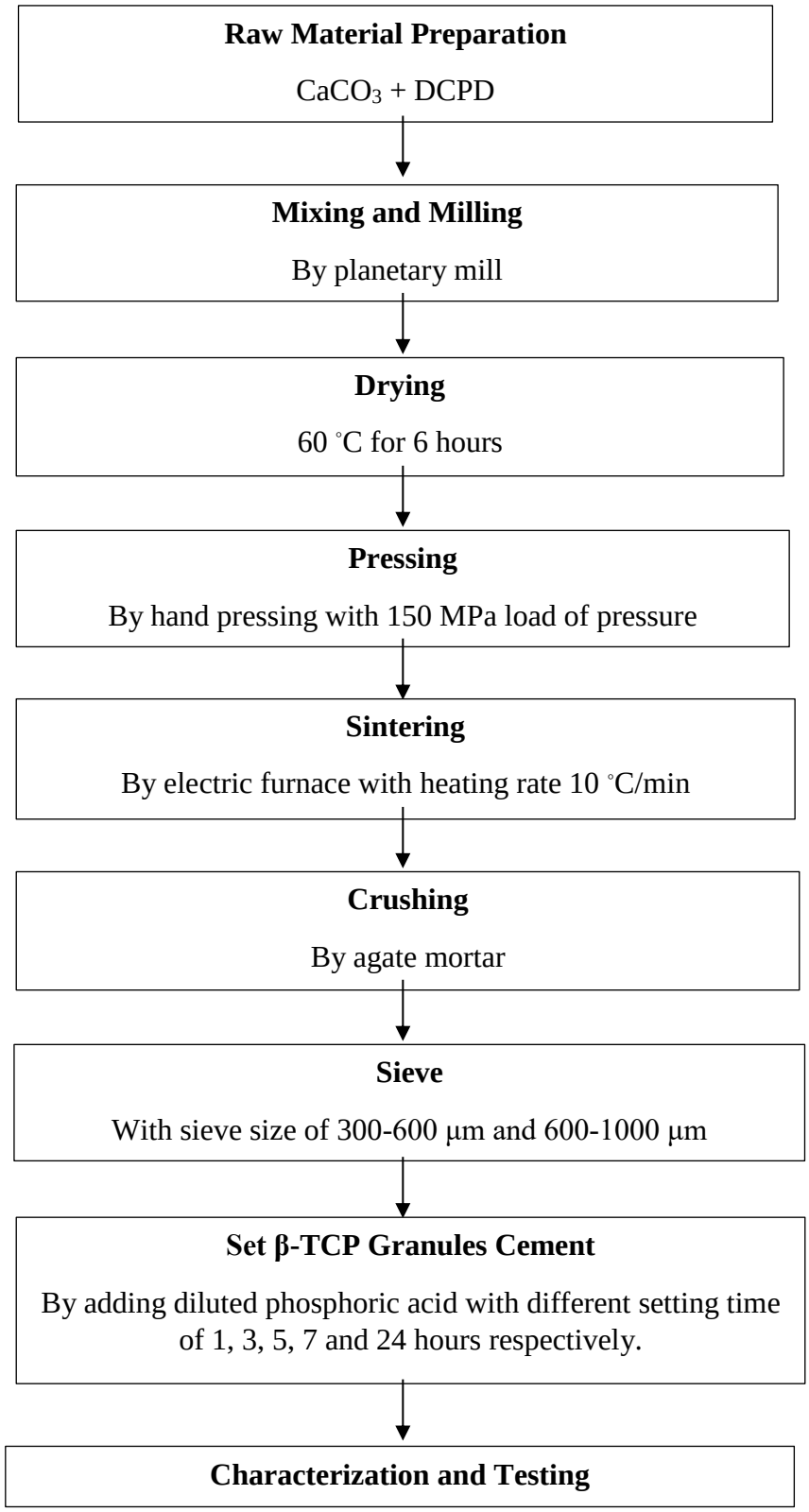

Table 1.1: Flowchart of the setting reaction process of β-TCP granules cement

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction on Calcium Phosphate as Biomaterials

Calcium phosphate presence in variability of chemical composition and structure. As it able to represent in a wide class of materials, calcium phosphate has been successfully used in many clinical applications. As an example, calcium phosphate formulation that proven to be efficient to develop clinical applications are Hydroxyapatite [HAp, $Ca_{10}(PO_4)_6(OH)_2$] and β-tricalcium phosphate [β-TCP, $Ca_3(PO_4)_2$] and (Mulongo-masamba et al., 2017).

Calcium phosphate currently being used as substitute to hard tissue due to its excellent bioactivity and capability when implanted in the bone defect area .Synthesis of hard tissue are usually made by using calcium phosphate as the main inorganic component (Carrodeguas & Aza, 2011). It is proven by (Shariff et al., 2015) that calcium phosphate able to help in cellular attachment by its ability to absorb the proteins spontaneously from the surrounding body fluid.

Calcium phosphate has been widely used in clinical applications as it shows good bioresorbility properties. In clinical applications, calcium phosphates are categorized in resorbable bioactive materials as shown in Figure 2.1 (Vallet-regi, 2001). Moreover, as calcium phosphate attached to living tissues it will then shows a positive interaction with each other. Hence, it will improve adhesion of ion in bone regeneration-meditating proteins at calcium phosphate surface as it has good chemical bond around bone and along the boundary of bone and cell (Al-Sanabani et al., 2013).

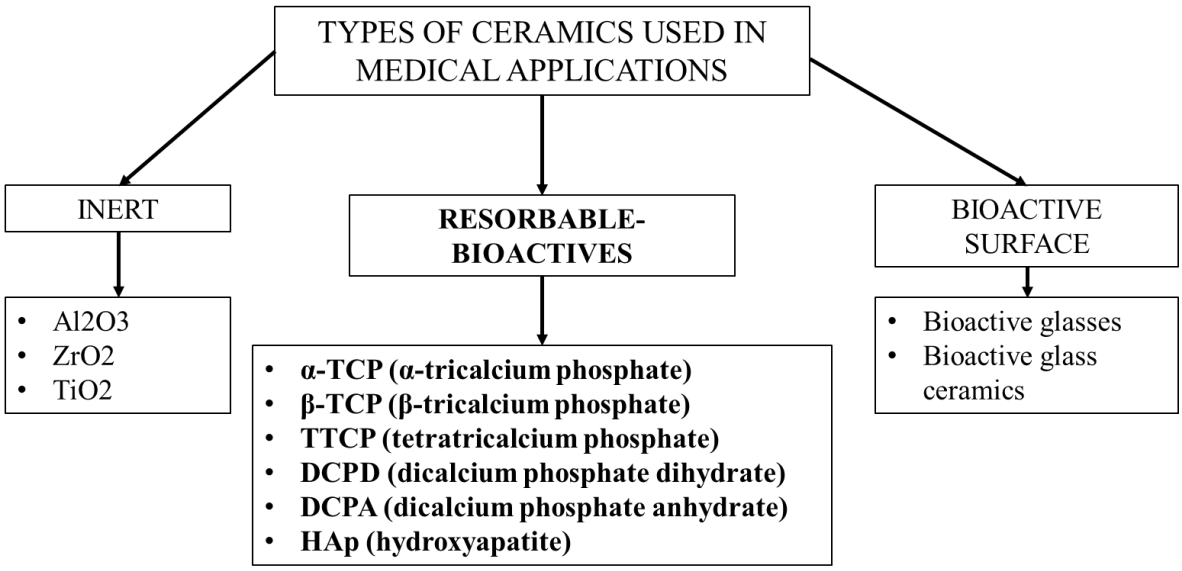

Figure 2.1: Classification of bioceramics in medical application (Vallet-regí, 2001)

2.2 Types of Calcium Phosphate

Calcium phosphate are an inorganic compounds that been differentiated by different stoichiometric amounts. Main constituent of calcium phosphate are Ca^{2+} and PO_4^{3-} ions. Calcium phosphate ceramics are categorized into certain type such as α-tricalcium phosphate (α-TCP), hydroxyapatite (HAp), dicalcium phosphate dihydrate (DCPD), anhydrous dicalcium phosphate (DCPA), tetracalcium phosphate (TTCP), β-tricalcium phosphate (β-TCP), calcium-deficient hydroxyapatite (HAp), and monocalcium phosphate monohydrate (MPCPM) (Driessens et al., 1998). Table 2.1 exhibiting the different type of calcium phosphate with their formula and calcium to phosphate ratio (Ca/P).

Table 2.1: Types of calcium phosphate (Driessens et al, 1998)

Ca/P	Calcium Phosphate	Formula
0.5	Monocalcium phosphate monohydrate MCPM	$Ca\ (H_2PO_4)_2.\ 2H_2O$
1.0	Dicalcium phosphate dihydrate (DCPD)	$CaHPO_4.2H_2O$
1.33	Octacalcium phosphate (OCP)	$Ca_3(HPO_4)_2\ (PO_4)_4.5H_2O$
1.5	Calcium deficient hydroxyapatite (CDHA)	$Ca_9\ (HPO_4)\ (PO_4)_5\ (OH)$
1.5	Tricalcium phosphate (TCP)	$Ca_{10}\ (PO_4)_6\ (OH)_2$
1.67	Hydroxyapatite (HA)	$Ca_{10}\ (PO_4)_6\ (OH)_2$

Generally in calcium phosphate, Ca/P ratio of materials are usually in between 0.5 to 2. But in clinical application it is preferable to used tricalcium phosphate (TCP) and hydroxyapatite (HAp) as it has Ca/P ratio from 1.5 and 1.67. Other significant consideration in calcium phosphate is it particles size (Bizari et al., 2016.)

TCP and HAp are desirable to be used in biocompatible calcium phosphate due to its biocompatibility, bioresorbility, and bioactive. HAp is a material that are thermodynamically stable in physiological environment, and able to perform a strong biological and chemical reaction with the living tissue once it attached with the surface of bone implant. This main reason is the composition of HAp contain main mineral in component of human bone. Whereas, β-TCP are also possible to be used as bone replacement as it also able to execute dissolution reaction when exposed to physiological environment (Pissiotis & Sp., 1990).

In bioceramics materials it is important that the material have bioresorbility properties. Generally resorbing rate is directly proportional to the calcium phosphate solubility, which is also affected by the pH (De et al., 2009). Solubility of different types of calcium phosphate materials are shown in Figure 2.2 (De et al., 2009).

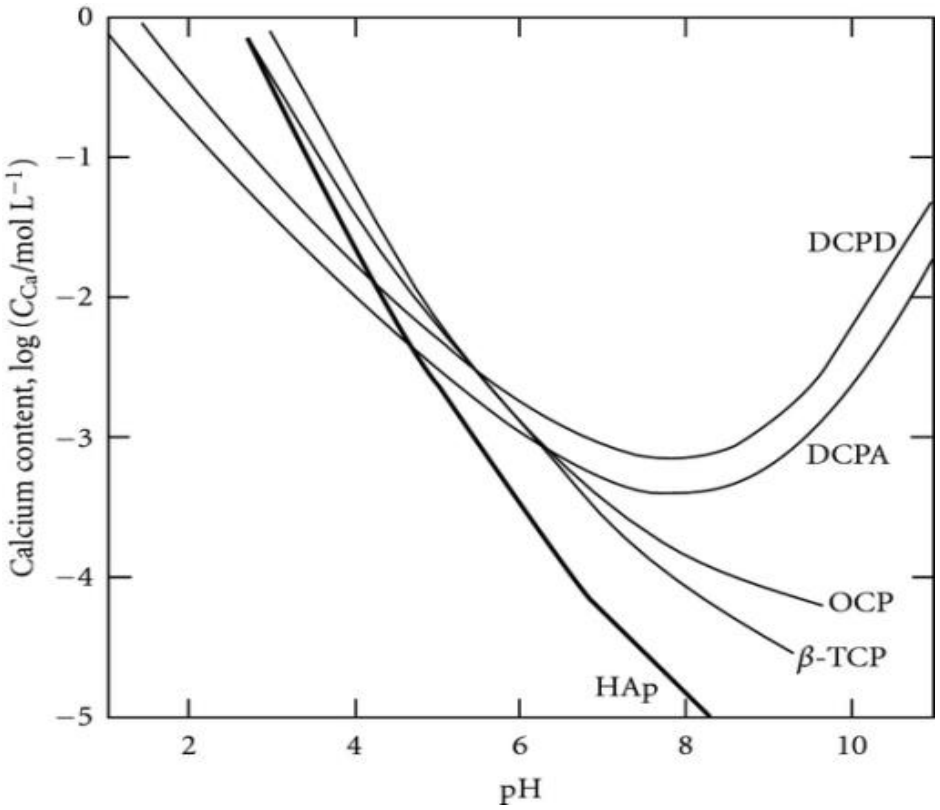

Figure 2.2: Solubility curves of calcium phosphate materials depending on pH in solution (De et al., 2009)

Figure 2.2 shows the solubility curves of various calcium phosphate materials. This figure indicates that DCPA is the most stable compound at pH <5, with HAp the most stable at pH >5. Therefore, HAp can be easily obtained in a solution where pH >5 and where the ion content and temperature is controlled. However, HAp cannot precipitate in a solution

where pH <5 because in aqueous solution, precipitation that occur to support β-TCP bioactive compound does not occur (Kuroda & Okido., 2012).

2.2.1 Hydroxyapatite (HAp)

Hydroxyapatite (HAp) comes with chemical formula of $Ca_{10}(PO_4)_6(OH)_2$. Hydroxyapatite possess an outstanding biological compatible with living cells hence able to permit new bone formation and adhesion. Also, HAp are as widely recognized as the golden material for bone and teeth as it has properties similar to inorganic part of bone (Dapporto et al., 2016).

HAp are categorized within the family of apatite mineral due to its specific composition. HAp are greatly used and studied by researcher as it have the ability to form through an acid-base reaction (Ginebra et al., 2012). HAp is the most fit ceramics material for bone regeneration from the point of view of biocompatibility. HAp is chemically match with mineral portions of hard tissue. (E.g. calcium + phosphorus). Component of mineral structure in living body such as bone and teeth seems to have similarity with artificial as presented in Figure 2.3.

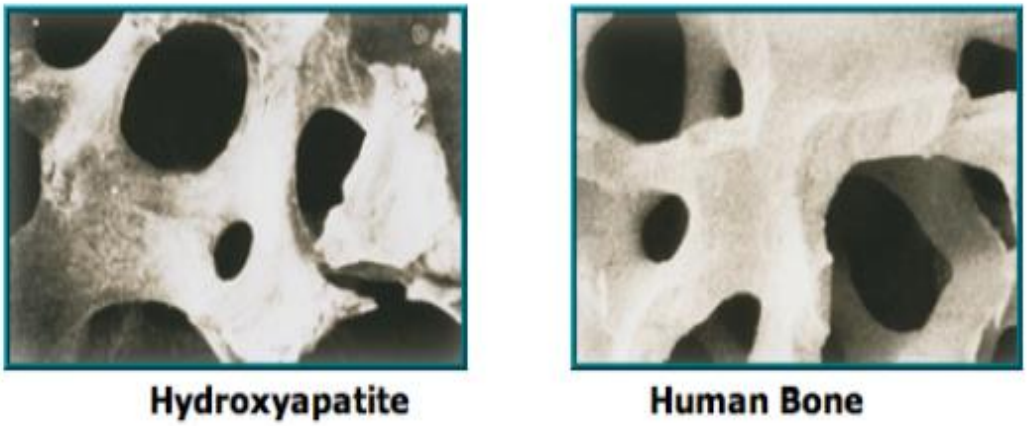

Figure 2.3: Comparison between hydroxyapatite and human bone (Sopyan et al., 2007)

HAp possess superb biological compatibility and proficient to stimulate growth of immature cells. Hence, due to its excellent properties usually HAp been used in bulk form, coating and cements (Yang et al., 2014). HAp can be categorized by its porous structure, phase, and handling method. As an outcome of tremendous favorable to be able stimulate growth of immature cell and biologically active within living cells, HAp are extensively been used as the high quality biomaterial either in orthopedics and dental applications (Al-Sanabani et al., 2013).

Through all advantages, HAp also has some disadvantages which it possess low strength when applied by mechanical force and low toughness when applications at load-bearing are applied (Al et al., 1998). Consequently the improvement in mechanical properties would broaden used of hydroxyapatite in clinical applications.

2.2.2 α-tricalcium Phosphate (α-TCP)

α-tricalcium phosphate (α-TCP, α-$Ca_3(PO_4)_2$) is considered as an excellent material used for several injectable hydraulic bone cements, biodegradable bioceramics and composites for bone repair (Frasnelli & Sglavo, 2016).

α-TCP normally formed through heating at temperature more than 1150 °C. As at temperature lower than that, formation occur only on β-TCP. α-TCP are known for its excellent solubility. In fact, α-TCP shows the highest solubility among other materials in calcium phosphate groups. Due to its good solubility and reactivity, α-TCP mainly used as fine powder to prepare calcium phosphate cement. Generally, α-TCP been used in as materials for self-setting osteotransductivity bone cement, biodegradable bioceramics and composites for bone repairing (Carrodeguas & Aza, 2011)

2.2.3 β-tricalcium Phosphate (β-TCP)

Usually β-TCP stable at low-temperature (<1150 °C). β-TCP possesses a rhombohedral crystal structure, related to whitlockite ($Ca_{18}Mg_2H_2(PO_4)_{14}$) (Frasnelli & Sglavo, 2016). β-TCP are having same chemical composition as α-TCP but differ in their structure, density and solubility which in turn determine their biological properties and clinical applications. β-TCP is mainly used for preparing biodegradable bioceramics shaped as dense and macro-porous granules and blocks.

β-TCP is attractive as bone substitutes due to their biocompatibility, biological safety, virtually unlimited availability, ease of sterilization, and long shelf life. β-TCP represents a good balance among absorption, degradation and new bone formation and can also preserve structural stability by releasing a large quantity of calcium ion (Ca^{2+}) and phosphate ion (PO_4^{3-}) ions, indispensable inorganic salts for new bone formation (Li et al., 2016).

β-TCP comes in many type which is scaffold, cement and granules. Commonly used as an artificial bone substitute is β-TCP granules since bone defect can be reconstructed by simply filling it with granules, resulting in good osteoconductivity and formation of new bone. However, the drawback is β-TCP granules often flow out from the defect. In previous study, by Alan et al., (2016) by using pure β-TCP ceramic granules to fill defect as shown in Figure 2.4. In Figure 2.5, the osteogenesis was observed between the granules and newly formed bone directly attached to the porous structure. Through this study, it can be conclude that β-TCP is a suitable materials for the filling of bone defects because of its versatility, low complication rate, and favorable long-term result. The lack of foreign body response or toxicity supports the usefulness of implant as bone substitute in bone transplant to repair defect.

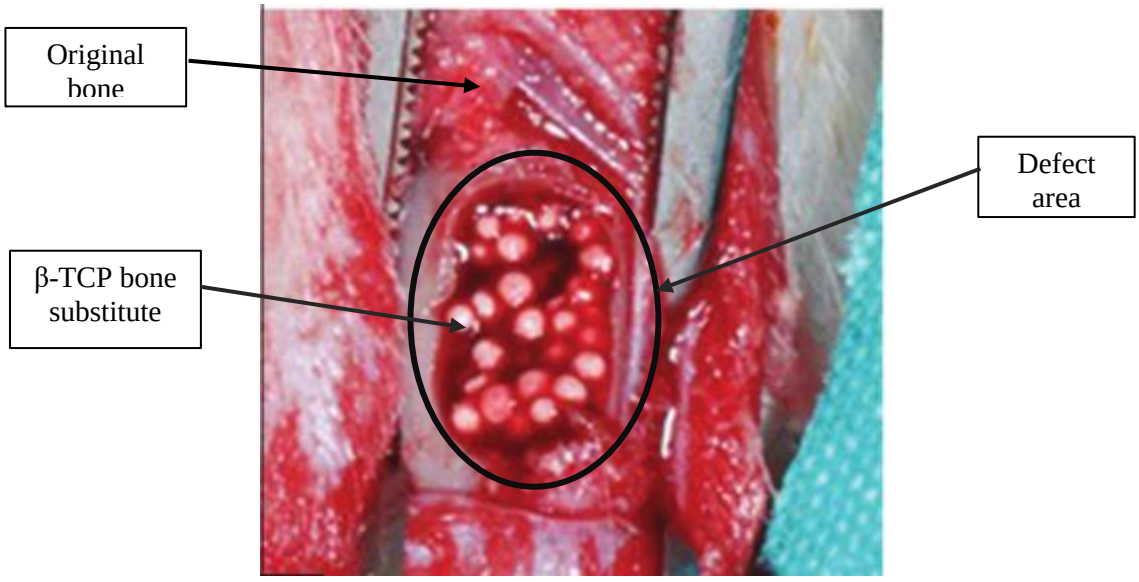

Figure 2.4: View of β-TCP filled in the defect (Alan et al., 2016)

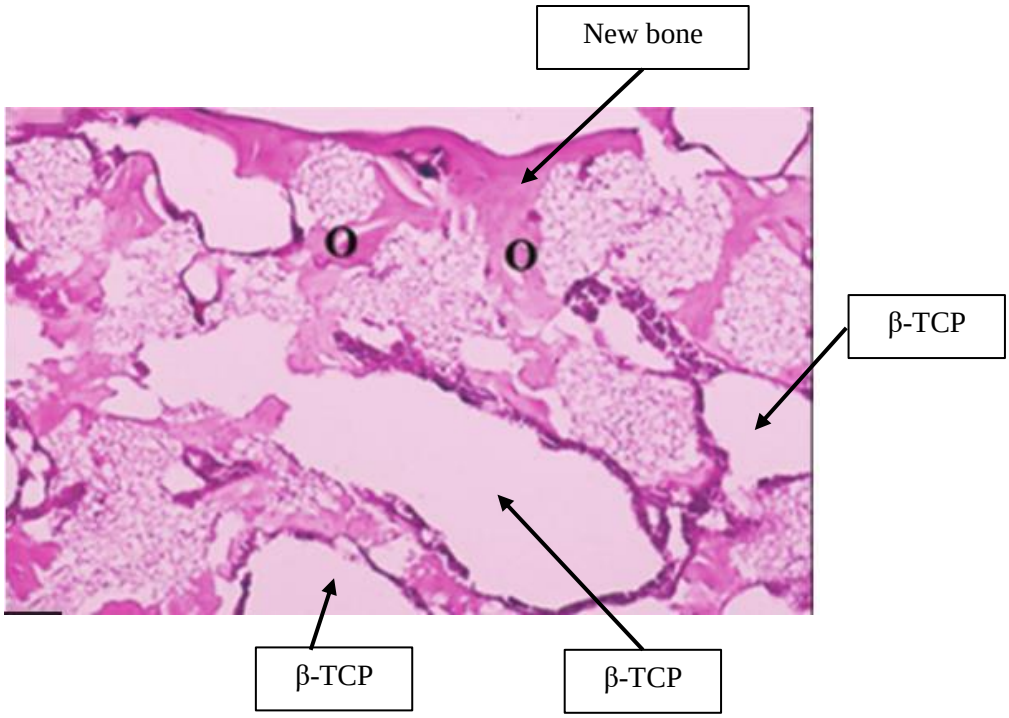

Figure 2.5: Photomicrograph shows growth of living tissues by β-TCP (Alan et al., 2016)

2.2.4 Dicalcium Phosphate Dihydrate (DCPD)

Dicalcium phosphate dihydrate (DCPD), with chemical formula ($CaHPO_4.2H_2O$) acknowledged as important mineral structures that present in both normal and pathological calcifications. Usually formation of DCPD occur in weakly acidic aqueous environment with pH value around 4-6 (Li et al., 2014). Also, DCPD is metastable under physiological conditions, and for this reason DCPD cement resorbs much faster than hydroxyapatite.

DCPD cement were obtained as a result of an acid–base reaction. Several compositions have been proposed for brushite cements, most of them containing β-tricalcium phosphate (β-TCP) and an acidic component, namely monocalcium phosphate monohydrate (MCPM) or phosphoric acid (β-TCP + MCPM, β-TCP + H_3PO_4), and (TTCP + MCPM+ CaO) (Ginebra et al., 2012).

2.3 Calcium Phosphate for Clinical Applications

Calcium phosphate has been used in clinical in order to repair periodontal defects, augmentation of alveolar bone, sinus lifts, tooth replacement, and repair of large bone defects caused by tumors. It also used as scaffolds in tissue engineering for bone or dentin regeneration. In addition it also used in the practice of injectable cements. Other than that calcium phosphate widely used as coatings. Other application used by calcium phosphate are as shown in Table 2.2.

Table 2.2: Summarize the use of calcium phosphate for clinical application

Compounds	Applications
Monocalcium phosphate monohydrate (MCPM)	Calcium phosphate cement (CPC) for orthopedic use (Nasri et al., 2015).
Dicalcium phosphate dehydrate (DCPD)	Bone substitute or bone filler for dental applications (Bizari et al., 2016).
Octacalcium phosphate (OCP)	Enhance bone regeneration and could be a good candidates for an advanced materials compatible to autologous bone or bone marrow (Suzuki et al., 2013).
α-tricalcium phosphate (α-TCP)	Repair and augmentation of osseous tissues and successfully used in several clinical applications such as dentistry, maxillofacial, orthopedic and spinal surgeries (Cicek et al., 2011).
β-tricalcium phosphate (β-TCP)	Scaffold that are effective in tissue-engineered bone regeneration (Li et al., 2016).
Amorphous calcium phosphate (ACP)	First commercial product as artificial hydroxyapatite used in dentistry application (Zhao et al., 2011).
Hydroxyapatite (HA)	Bone tissue regeneration, cell proliferation, and drug delivery (Sopyan et al., 2007).

2.4 Different Types of β-TCP

2.4.1 β-TCP Scaffolds

Bone tissue engineering has been recognized as a promising approach for bone repair and reconstruction. The scaffold is one of the key elements of bone tissue growth before gradually degrading and getting eventually replaced by the new tissue. For regeneration of large and complex bone tissue, the scaffold must possess several structural features: precise control of scaffold porosity and architectural parameters (e.g. pore geometry, size, interconnectivity, orientation and branching), which are necessary to maximize nutrient diffusion and interstitial fluid flow to control cell growth and function.

Scaffolds are the main component for bone tissue engineering. HAp are widely work as template for cell attachment and proliferation. Scaffold must come with interconnected porous structure that have more than 100-200 μm of pore size. This range of pore size is the most appropriate size that would permit and encourage cell penetration. Other than that, out of all parameters, mechanical strength of scaffold must be good enough so that scaffold able to sustain mechanical stability when cells attachment form a new cells around the graft.

In scaffold, properties of porosity and strength in mechanical are opposite with each other. When scaffold have high porosity its mechanical strength will reduced. In contrast, scaffold with low porosity will have high mechanical strength. This situation can be solved by adding or coating the scaffold with biopolymer materials. The biopolymer filler will prevent scaffold from failure if mechanical strength applied in the scaffold. Peroglio et al reported that polymer layer created on the surface of scaffold works to fill cracks and voids.

This conditions will improve mechanical stability. The biopolymer materials actually work to stabilize mechanical strength by induce crack bridging mechanism.

In order to approach bone graft that are compatible to natural bone, this method of adding biopolymer as filler are used. Natural bone, have composition of 60% of HAp and 40% of collagen and water. It is expected by adding biopolymer into porous scaffold will improve its mechanical strength (Amyra et al., 2016)

2.4.2 β-TCP Cements

β-TCP cements usually produced by mixing one or more calcium phosphate. By mixing it with liquid phase such as water and aqueous solution it will then form a paste that able to set and harden. The cement able to set and harden through process of dissolution and precipitation.

Generally, end product of tricalcium phosphate cement come only in two types which is precipitated HAp and DCPD as stated in details as in Figure 2.6 (Ginebra et al., 2012). This can be explained by the fact that, at pH more than 4.2 HAp is in the most stable form while at pH lower than 4.2 brushite are in it stable form. However, β-TCP will form brushite as end product through acid base reaction by adding acid such as monocalcium phosphate monohydrate (MCPM) as shown in Figure 2.6.

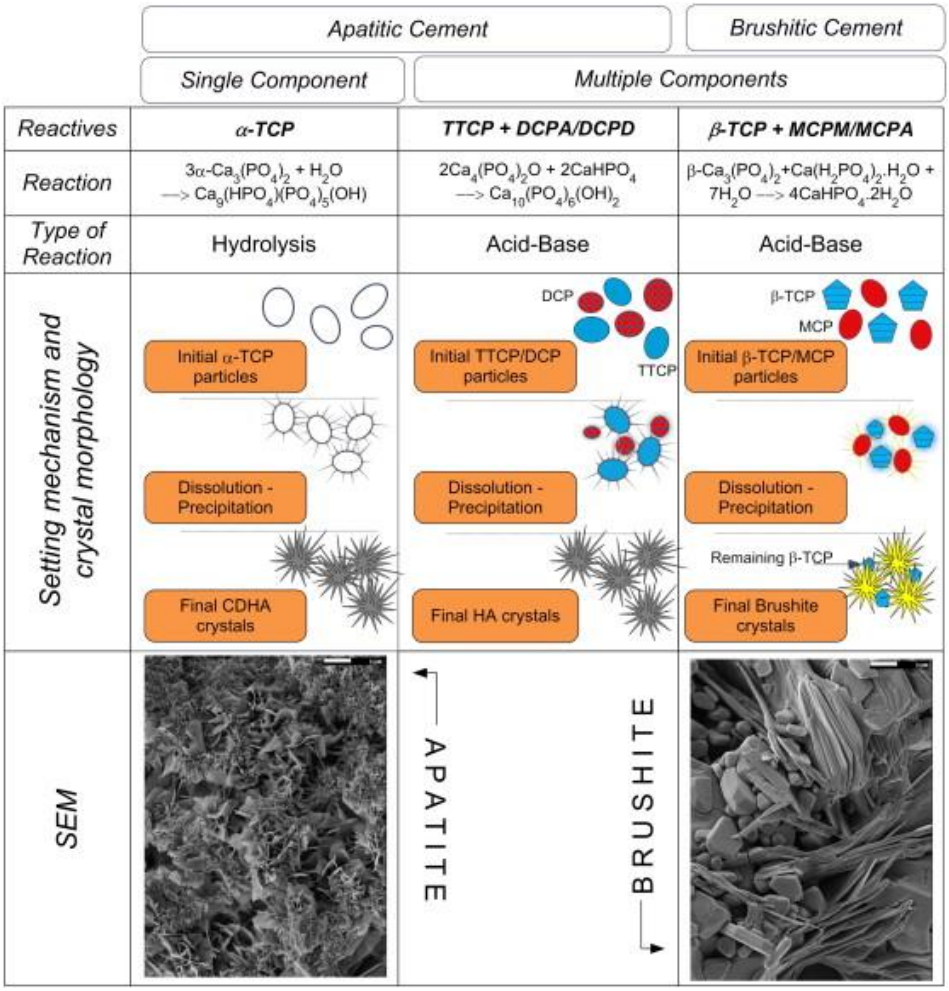

Figure 2.6: Classification of calcium phosphate cement (Ginebra et al., 2012)

Figure 2.6 shows example of the most common formulations that classified type of calcium phosphate cements. From top to bottom the cements are classified by the type of end-product either hydroxyapatite (HAp) or dicalcium phosphate dihydrate (DCPD), number of components in the solid phase either single or multiple, type of setting reaction either hydrolysis or acid–base reaction, setting mechanism and microstructure evolution during setting. Scanning electron micrographs of set apatite and brushite cements obtained by the

hydrolysis of α-TCP and by reaction of β-TCP with MCPM (monocalcium phosphate monohydrate) respectively, are also shown (Ginebra et al., 2012).

There are three main setting reaction stages in order to form β-TCP cement. First stage is dissolution of the reactant then followed by nucleation of the new phase of brushite and lastly is crystal growth (Chen et al., 2002). In other word, the setting reaction basically done through dissolution–precipitation process. In the first stage which is dissolution of the reactant, the reaction will generate a supersaturation level in the solution as calcium phosphate will discharge calcium (Ca^{2+}) and phosphate (PO_4^{3-}) ions. Next stage is nucleation of new phase. Nucleation will occurs when Ca^{2+} and PO_4^{3-} ions reach a supersaturated level. Nucleation formation will take place surrounding powder particles. At final stage, the new phase that previously formed will keep growing as long as the dissolution of the reagents goes on. For the period of the first hours, the setting process is controlled by the dissolution process of the raw materials, but once the new phase surrounds the reactants, the process is controlled by diffusion across the new phase.

β-TCP cement usually formed with porous structure. Porosity appear along the gap between precipitated crystals. Commonly, presence of pore size were in micrometer size (Espanol et al., 2009). It is important to take porosity into consideration in formation of β-TCP cement as porosity give a good impact to enhance materials bioactivity and resorbability. By the presence of porosity in structure, exposure of large surface area for cell attachment occur. Proper amount of porosity allow materials to conduct a good carrier properties to the cell and at the same time improve bone remodeling process.

All these porosity factors will diverge depending on the processing settings which is restricted to liquid to powder (L/P) ratio and particle size as shown in tabulated Figure 2.7. As liquid to powder ratio arise the total porosity will also rise. Other than that, porosity also affected by size and shape particle of starting materials and similarly to shape and size of precipitated crystal (Ginebra et al., 2012).

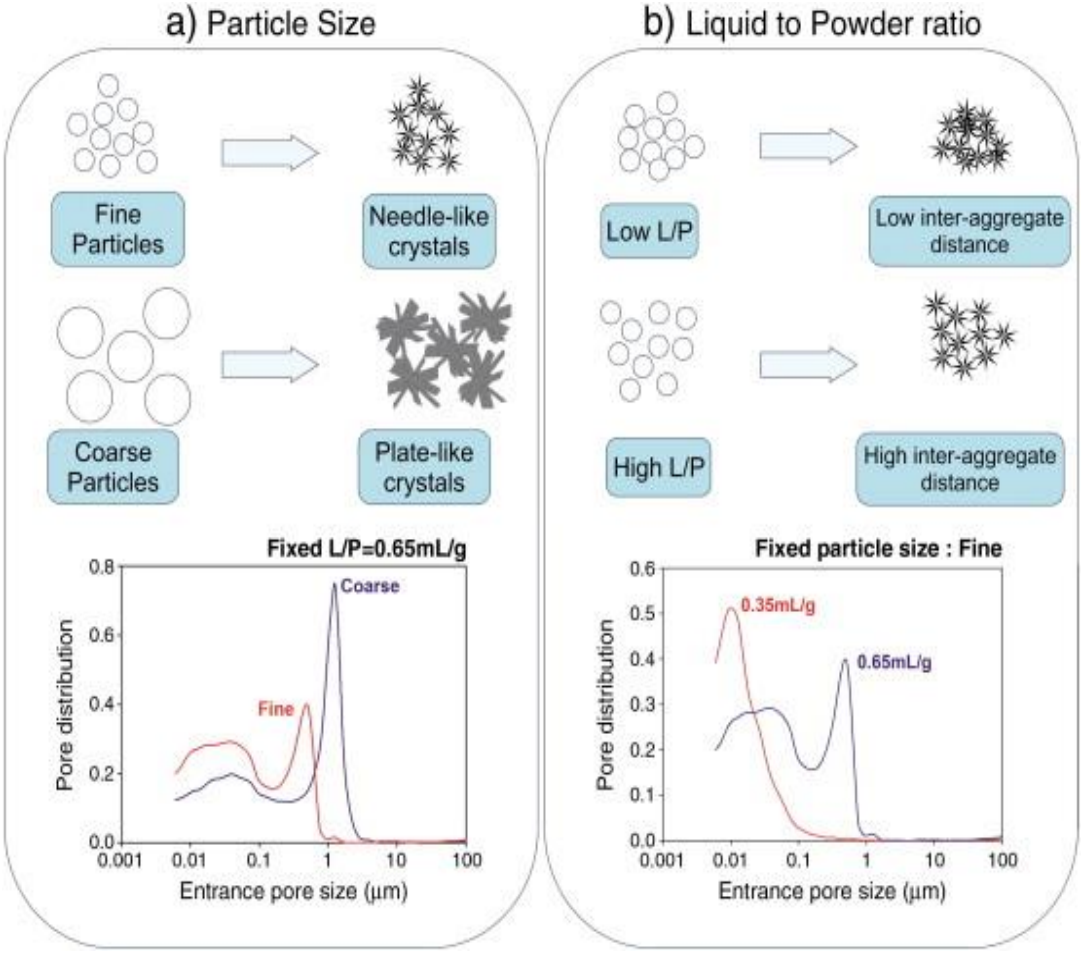

Figure 2.7: Condition of cement with varies particle size and L/P ratio (Ginebra et al., 2012)

Other than above parameter, calcium phosphate cement must own bioactivity properties. For that reason, in bone substitution bioactivity working to assist material so that it will bind directly with the surrounding bone without reacting to other living cell like macrophages or osteoclast. Furthermore, bioactivity properties works to avoid chemical dissolution by inactive resorption.

For DCPD it is identified that it is quickly dissolve in body fluids (Theiss et al., 2005, Grover et al., 2003). In demand to diminish degradation of calcium phosphate cement, the integration of ions substitution is used. Added, by monitoring parameter of porosity and crystallinity correspondingly can aid to modulate calcium phosphate resorption. New bone in vivo were progressively replaced once calcium phosphate resorbed. This occurrence happens in ideal conditions. As the resorption of the cement surface is simultaneously occur with bone growth, the bone replacement will also occur. This gap between living tissue and bone implant that were reduced will then guide new bone formation (Driessens et al., 1998).

2.5 Issues in β-TCP Cements

Commonly, β-TCP cements end product is dicalcium phosphate dihydrate (DCPD) which was obtained by mixing one or more calcium phosphate powders that reacted with acidic solution. Hence, as chemical reaction take place it will harden aqueous paste. It has been proven through in vivo studies, that β-TCP cement are generally biocompatible, osteoconductive and also bioresorbed (Marcantom, 2010).

However, β-TCP has some drawbacks. In cement, setting time play the most important parameters must take into consideration. Previous study by Fukuda et al., (2017) reported that, β-TCP cement were set approximately 1 minute. Able to set at fast setting time can be considered as an advantages to the studies. However, when applied in clinical applications the setting time is considered as inconvenient as the surgeon have difficulty to handle it during surgery. In contrast, if the setting time is too long, the surgery process will took too long than it should and in the same time would exposed the wound at longer time and increase the probability of infection. Thus, an intermediate setting time is the most suitable in cement bone implantation surgery. Setting time between 1 to 20 minutes are in good range. Superior setting time is within 2 to 15 minutes, in more specifics in the range of 5 to 12 minutes (Marcantom, 2010)

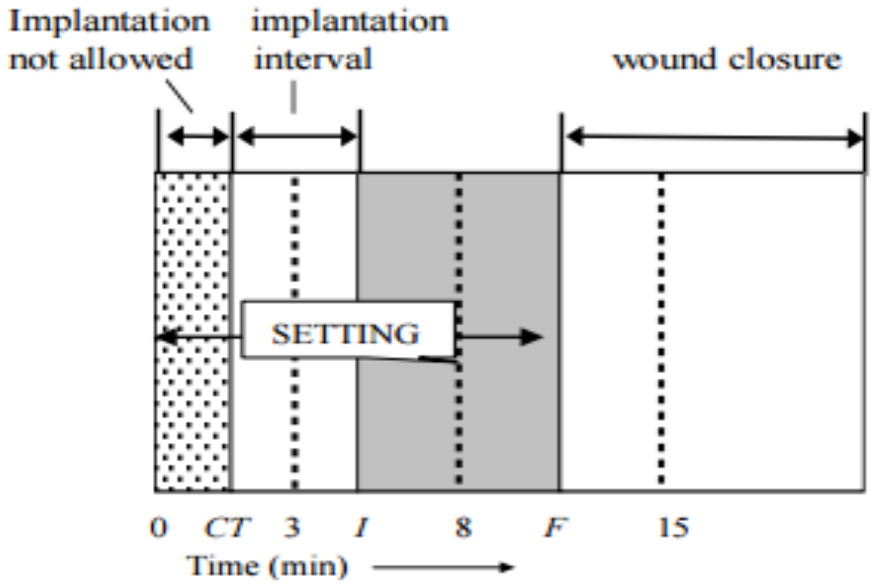

Figure 2.8: Ideal setting time for β-TCP granular cement (Dorozhkin, 2011)