EFFECT OF ACIDIC CALCIUM PHOSPHATE SOLUTION CONCENTRATION ON THE SETTING PROPERTIES OF POROUS β-TRICALCIUM PHOSPHATE GRANULAR CEMENT

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by

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DECLARATION

I hereby declare that I have conducted, completed the research work and written the dissertation entitled **"Effect of acidic calcium phosphate solution concentration on the setting properties of porous β-tricalcium phosphate granular cement".** 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 others examining body or university.

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Date : 25 JUNE 2018

Witnessed by

Supervisor : Dr. Khairul Anuar bin Shariff Signature

Date : 25 JUNE 2018

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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
ICDD	International Center for Diffraction Data
L/G ratio	Liquid to granule ratio
L/P ratio	Liquid to powder ratio
PSA	Particle size analysis
SEM	Scanning electron microscopy
FESEM	Field emission scanning electron microscopy
XRD	X-ray diffraction
Mag	Magnification

LIST OF SYMBOLS

α	Alpha
θ	Theta
μ	Micron
◦	Degree
μm	micro meter
mol/L	Molar/liter
ml	Milliliter
g	Gram
rpm	Rotation per minute

KESAN KEPEKATAN LARUTAN ASID KALSIUM FOSFAT TERHADAP SIFAT PENGERASAN SIMEN β-TRIKALSIUM FOSFAT GRANUL BERLIANG

ABSTRAK

Tujuan kajian ini adalah untuk menghasilkan simen β-trikalsium fosfat granul berliang dengan mendedahkan β-TCP granul (300-600 μm) bersama kepekatan larutan asid kalsium fosfat yang berlainan. Bagi mencapai objektif ini, β-trikalsium fosfat granul didedahkan kepada 1.0 mol/L larutan asid fosforik 1 mol/L H_3PO_4-0.2 mol/L, 1 mol/L H_3PO_4-0.8 mol/L dan 1 mol/L H_3PO_4-1.4 mol/L MCPM dengan nisbah larutan terhadap granul 1ml:1g. Disamping itu masa pengerasan yang digunakan dalam kajian ini ditetapkan pada 1,5,10,30 dan 30 minit. Spesimen yang telah mengeras akan dicirikan menggunakan XRD bagi mengesan pembentukan fasa dikalsium fosfat dihidrat (DCPD) di dalam spesimen yang telah mengeras. Didapati bahawa semakin tinggi kepekatan larutan asid kalsium fosfat, semakin tinggi dikalsium fosfat dihidrat yang terhasil. Hasil ini adalah konsisten dengan keputusan XRD dimana jumlah DCPD untuk 1 mol/L H_3PO_4-1.4 mol/L MCPM adalah lebih tinggi berbanding dengan 1 mol/L H_3PO_4-0.2 mol/L MCPM. Keputusan SEM menunjukkan banyak kristal DCPD berhubung silang diantara satu sama lain apabila kepekatan asid kalsium fosfat ditingkatkan. Jumlah kristal DCPD berhubung silang, yang tinggi akan meningkatkan kekuatan mampatan bagi spesimen yang mengeras. 14 mol/L MCPM-1 mol/L H_3PO_4 menunjukkan keliangan yang terendah iaitu 74.5% dan kekuatan mampatan yang tinggi iaitu 5.86 MPa jika dibandingkan dengan kepekatan yang lain. Justeru, ini menunjukkan bahawa peningkatan kepekatan larutan asid kalsium fosfat akan memberi kesan kepada sifat

pengerasan bagi simen β-trikalsium fosfat granul berliang iaitu pengerasan simen seawal 5 minit yang mana ia di dalam sengka masa yang sesuai.

EFFECT OF ACIDIC CALCIUM PHOSPHATE SOLUTION CONCENTRATION ON THE SETTING PROPERTIES OF POROUS β-TRICALCIUM PHOSPHATE GRANULAR CEMENT

ABSTRACT

The purpose of this study is to fabricate porous β-tricalcium phosphate granular cement by exposing β-TCP granules (300-600 μm) with different concentration of acidic calcium phosphate solution. β-tricalcium phosphate granules were exposed to 1.0 mol/L phosphoric acid, 1 mol/L H_3PO_4-0.2 mol/L, 1 mol/L H_3PO_4-0.8 mol/L and 1 mol/L H_3PO_4-1.4 mol/L MCPM with liquid to granular ratio (L/G) of 1:1 respectively. Besides, the setting time used in this study were fixed at 1,5,10,30 and 50 minutes respectively. The set specimen was characterized by XRD to detect the formation of dicalcium phosphate dihydrate (DCPD) phase in the set specimen. It was found that when high concentration of acidic calcium phosphate solutions was used, more DCPD formation were observed. This finding consistent with XRD result which the DCPD amount for 1.4 mol/L MCPM -1.0 mol/L phosphoric acid is higher than the 0.2 mol/L MCPM -1.0 mol/L phosphoric acid. SEM result demonstrated that more DCPD crystal were interlocked each other when increased concentration of acidic calcium phosphate solution. The high amount of interlocked DCPD crystal will increased the compression strength of set specimen. 1.4 mol/L MCPM -1.0 mol/L phosphoric acid shows the lowest porosity which is 74.5% and high compression strength which is 5.86 MPa in comparison with other concentration. Therefore, it shows that increasing of acidic calcium phosphate solution concentration will affected the setting properties of porous β-tricalcium phosphate

granular cement which the cement is fully set at 5 minutes which in the range of ample time.

CHAPTER 1

INTRODUCTION

1.1 Background of Research

Calcium phosphate based biomaterials are now used in a number of different applications throughout the 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 and 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/or replaced as the body rebuilds the damaged tissues. One of the well-known bioceramics materials is β-tcp. The main driving forces 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 phosphate, 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-Tricalcium phosphate is suitable to be used as their Ca/P ratio is 1.50.

Previous study has reported that interconnected porous cements were fabricated by exposing α-TCP foam granules with saturated acidic calcium phosphate solution (Shariff et al, 2016). However, the setting time found in this study is difficult to control for clinical application. Since chemical formula of α-tcp 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. However, this study also face similar problem which setting time issue (Fukuda *et al.*, 2017). 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.*, 2018).

1.2 Problem Statement of Research

β-TCP cement has widely used in clinics and hospitals because it shows good osteoconductivity and biocompatibility after implanted in the body. However, β-TCP cements shows dense structure after setting in the body. The reason is that, for β-TCP cement currently β-TCP powder used as starting materials. Due to the small size of the powder, the dense structure is expected to form which prevents cell penetration inside the β-TCP cements.

To overcome this issue, porous β-TCP cement has been introduced by other researcher (Fukuda *et al.*, 2018). For example, Wu *et al*, (2013) mixed β-TCP powder with porosity agent such as PMMA or fibre-glass in order to create porous β-TCP

cements. Meanwhile, Kucharska *et al.* (2012) have introduced the mixture of chitosan solution and sodium bicarbonate in order to fabricate porous structure. Recent study done by Fukuda *et al.*, (2018) found that, porous β-TCP cement can be produced by exposing β-TCP granules with supersaturated acidic calcium phosphate solution. However, it has been found that the setting time of the porous β-TCP cement is too fast which is 1 minute.

For surgical procedure, the ample time to allow the cement to set is between 5-12 minutes (Martin, 2006). Due to the applications, the ample time is needed in order to prevent the wound to be affected by others things. Therefore, based on this requirement, Fukuda *et al.*, (2018) study can be improved. In this study, the setting time of β-TCP granular cements could be regulated by modifying the concentration of acidic calcium phosphate solution. It is expected that increasing of concentration of acidic calcium phosphate solution will increase the setting time of β-TCP granular cement.

1.3 Objectives of Research

1. To fabricate β-tricalcium phosphate granules by using calcium carbonate and dicalcium phosphate dihydrate powder as starting materials.
2. To investigate the effect of different concentration of acidic calcium phosphate solution concentration on the setting properties of β-TCP granular cements

1.4 Scope of Research Work

The main materials used in this study is β-tricalcium phosphate. This β-TCP was obtained by mixing calcium carbonate ($CaCO_3$) and dicalcium phosphate dihydrate (DCPD) with a Ca/P ratio of 1.5. Both raw materials were mixed by using planetary mill to obtain homogenous mixture. Then the raw materials were dried in oven for 6 hours at 60 °C. After drying process, the powder is then pressed in the form of pellet. Then the pellet was sintered by using furnace at 1100 °C for 6 hours. After that, pellets were crushed by using agate mortar and sieved to obtain the required granules size which is 300-600 μm. Next, these granules were placed into the mold with 6 mm diameter and 3 mm height. Then, the three different concentration solution of acidic calcium phosphate solution was dropped into the mold accordingly with liquid to granular ratio 1 ml:1 g at 25 °C. 1 mol/L of phosphoric acid solution were also dropped into the mold as a control. The setting times were used in this study are 1,5, 10, 20, 30, 40 and 50 minutes respectively. The set specimen was washed with acetone to stop the setting reaction. Finally, the specimens were characterized accordingly.

To verify the raw materials used in this study X-Ray Diffraction (XRD) and Field Emission Scanning Electron Microscope (FESEM) were used to observe certain characteristics which are phase composition, morphology and elemental analysis.

The porous β-TCP granular cement was subjected to many characterization tests such as appearance observation, porosity and bulk density, compressive strength test, peak area ratio, Field emission scanning electron microscope (FESEM), X-Ray diffraction (XRD) and Energy Dispersive X-Ray (EDX). Those testing were used to

analyzed the microstructure, phase and the compositions of the porous β-tricalcium phosphate granular cement.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction of calcium phosphate as biomaterials.

Calcium phosphate presence in varies of chemical structure and composition. It is also has been widely used successfully in surgical and clinical applications. This had been proved that the calcium phosphate based materials which is efficient to develop clinical applications are β-tcp and hydroxyapatite. (Mulongo-Masamba *et al.*, 2017). Currently, calcium phosphate is being used as bone substitute for hard tissue because it has a good bioactivity and biocompatibility during the implantation in the defected bone. Previous study by Shariff *et al.* (2016) had proved that calcium phosphate is able to help in attachment of cellular due its ability to absorb the proteins spontaneously from the surrounding of body fluid.

The calcium phosphate has been widely used recently because it has good bioresorbility properties. Calcium phosphate can be classified in resorable bioactive materials especially in clinical application as shown in Figure 2.1. In addition, as long as the calcium phosphate attached to living tissues then it will show a positive interaction each other. Hence, it will improve adhesion of ion in bone regeneration-meditating proteins at the surfaces of calcium phosphate. It because it has good chemical bond

around the bone. It also has good chemical bond along the bone's boundary and cell. (Al-Sanabani et al. 2013).

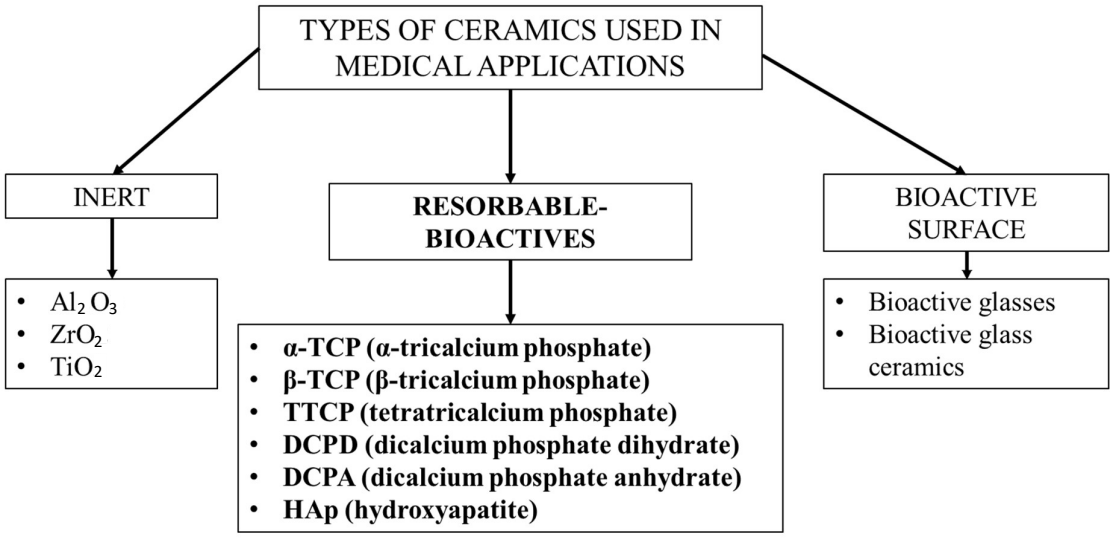

Figure 2. 1: The classification of bioceramics in medical application (Vallet-regí ,2001)

2.2 Calcium phosphate for clinical application

Calcium phosphate could be used in clinical application. It also could be used in dental implantation and also could fix bone defect in large scale. Furthermore, calcium phosphate also used as scaffold tissue in formation of tissue specifically for bone regeneration. Moreover, it also had been used in the practice of injectable cements. Other applications used by calcium phosphate are shown in Table 2.1 since it shows various properties such as able to self-set, easily shape, biological compatible and no residue in body. Calcium phosphate is known to be used as bone substitutes.

Table 2.1: The summary of calcium phosphate usage

Types of calcium phosphate	Clinical application
Monocalcium phosphate monohydrate (MCPM)	Calcium phosphate cement (CPC) for orthopedic usage. (Nasri *et al.*, 2015)
Dicalcium phosphate dihydrate (DCPD)	Use as bone substitute or bone filler in dental applications (Bizari *et al.*, 2016)
Octacalcium phosphate (OCP)	Enhance bone regeneration and could be good candidates for an advanced materials compatible to autologous bone or bone marrow. (Suzuki, 2013)
α-tricalcium phosphate (α-TCP)	Use as raw materials for several injectable hydraulic bone cements, biodegradable bioceramics and composites for bone repair (Carrodeguas *et al*, 2011)
β-tricalcium phosphate (β-TCP)	β-TCP composite materials have been used in the field of orthopaedics which use for bone repairing materials and bone substitute.(Liu *et al*, 2012)
Amorphous calcium phosphate (ACP)	First commercial product as artificial hydroxyapatite used in dentistry application (Zhao *et al.*, 2011)
Hydroxyapatite (HAp)	Hydroxyapatite commonly used as bone grafts, fillers and as coatings for metal implants. (Mucalo, 2015)

2.3 Types of calcium phosphate

Calcium phosphate are inorganic compound which that has been differentiated by a different of stoichiometry which it main constituent of calcium phosphate ions are Ca^{2+} and PO_4^{3-} ions. Previous research, done by Driessens *et al.* (1998) mentioned that the calcium phosphate ceramics had been categorized into different types such as α-tcp, β-tcp, HAp, DCPD, anyhydrous dicalcium phosphate and MCPM. Table 2.2 below shows the different types of calcium phosphate with their chemical formula and Ca/P ratio.

Table 2.2: Types of calcium phosphate with their chemical and Ca/P ratio(Driessens *et al.*, 1998)

Ca/P Ratio	Calcium Phosphate	Chemical Formula
0.5	Monocalcium phosphate monohydrate (MCPM)	$Ca(H_2PO_4)_2.2H_2O$
1.0	Dicalcium phosphate dehydrate (DCPD)	$CaHPO_4.2H_2O$
1.33	Octacalcium phosphate (OCP)	$Ca_3(HPO_4)_2\ (PO_4)_4.5H_2O$
1.5	Tricalcium phosphate (TCP)	$Ca_{10}\ (PO_4)_6\ (OH)_2$
1.67	Hydroxyapatite (HAp)	$Ca_{10}\ (PO_4)_6\ (OH)_2$

The Ca/P ratio between 0.5 to 2 are general for the calcium phosphate but referring to researchers (Bizari *et al.*, 2016), for clinical application it is preferable to used tricalcium phosphate (TCP) and hydroxyapatite (HAp) due to their Ca/P ratio 1.5 and 1.65 respectively. Moreover, other significant consideration in calcium phosphate is it particle size because the size will effected the density (Bizari *et al.*, 2016).

Due to their properties in clinical application, tricalcium phosphate and HAp are used in the biocompatible calcium phosphate. β-tcp is possible to be used as bone replacement which it also capable to execute dissolution reaction when it exposed to physiological environment. Moreover, β-tcp are osteoconductive. It is because osteoblasts adhere on them and deposit bone tissues on their surfaces (Horowitz *et al.*, 2010). For the hydroxyapatite, it is a material that are thermodynamically stable also in physiological environment which it took long time to be dissolved in the body Kuroda & Okido (2012). The main thing is composition of hydroxyapatite itself already contain main mineral as in human bone component. (Pissiotis and Spngberg, 1990). Bioresorbility properties play important role in bioceramic materials. Resorbing rate is directly proportional to the calcium phosphate which it is also affected the pH value. This is proved by Kuroda & Okido (2012) and (Augusto *et al.* 2017) in their researches.

The solubility curve for calcium phosphate compounds at 37 °C which is depending on the pH value. From the Figure 2.2 above it shows that DCPD are stable at pH<5. At pH>5 found that HAp is the most stable. Moreover, HAp stable at the pH>5 and where the temperature and ion content are being controlled. Referring to the research, at pH<5, HAp cannot form precipitation reaction which is this reaction is most important in clinical application.

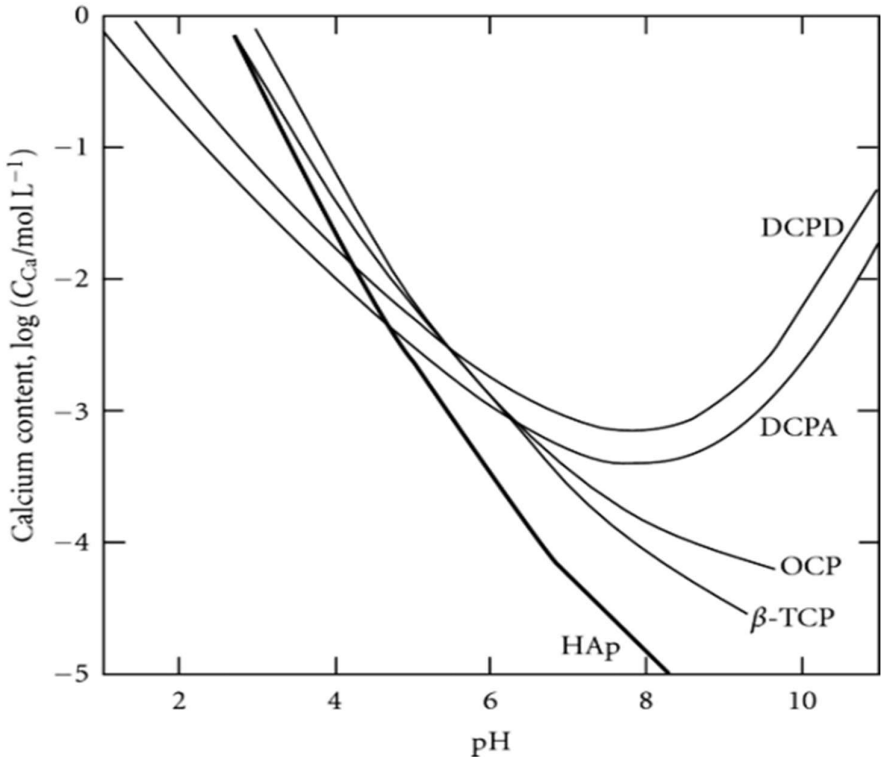

Figure 2.2: Solubility curve for CaP materials (Kuroda & Okido, 2012) (Augusto *et al.*, 2017)

2.3.1 Monocalcium Phosphate Monohydrate (MCPM)

Monocalcium phosphate monohydrate (MCPM) also known as calcium dihydrogen phosphate with Ca/P ratio 0.5 which has low solubility. Chemical structure is shown in Figure 2.3. MCPM can be dissolves in water and it will release $Ca^{2+,}$ PO_4^{3-} and H^+ ions. Moreover, MCPM has low solubility properties. MCPM acts as hydraulic binder which is a mixture of substances which not stable in the presence of water. It also released proton resulting phosphate ions and then the solution become DCPD precipitation (Mirtchi *et al*, 1989). The investigations about MCPM continued, by further

evaporation of the solution and the result is MCPM was contaminated with excess phosphoric acid (Nasri *et al.*, 2015)

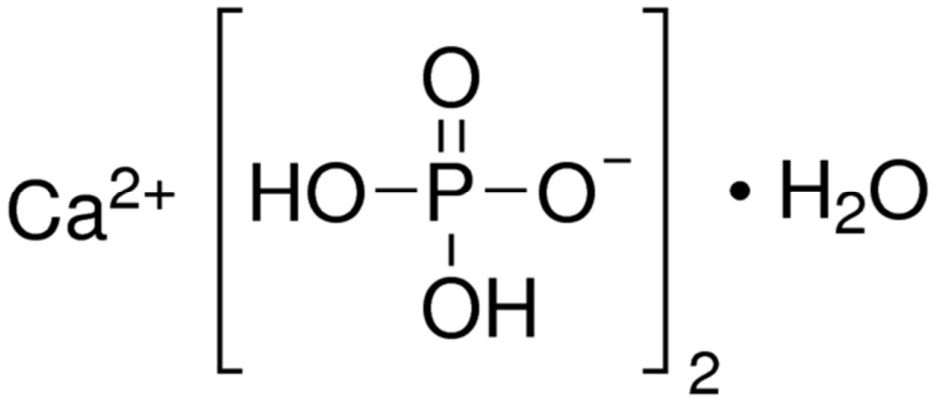

Figure 2.3: Monocalcium phosphate monohydrate structure (Chen *et al.* 2013)

2.3.2 Dicalcium Phosphate Dihydrate (DCPD)

Dicalcium phosphate dehydrate (DCPD) has chemical formula ($CaHPO_4.2H_2O$). The chemical structure is shown in the Figure 2.4. Refer to the research, formation of DCPD usually occur at pH value around 4-6 in aqueous condition (Li *et al.*, 2014). In addition, DCPD also have higher resorbability than hydroxyapatite and in physiological condition it is more stable.

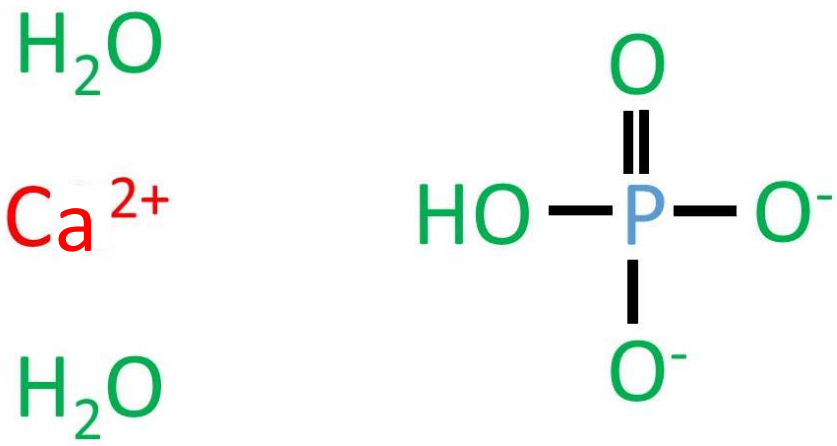

Figure 2.4: Dicalcium Phosphate Dehydrate structure (Frasnelli and Vincenzo M. Sglavo, 2016)

From the acid base reaction, DCPD was produce. In order to produce DCPD cement, lot of mixture of composition is suggested. For example β-TCP with monocalcium phosphate dehydrate. Actually that mixture is come from the mixture of β-TCP with acid solution theoritically. (Ginebra *et al.*, 2012).

2.3.3 Octacalcium Phosphate (OCP)

Octacalcium phosphate (OCP) which have chemical formula $Ca_3(HPO_4)_2(PO_4)_4.5H_2O$ and chemical structure as shown in Figure 2.5. It is one of the osteoinductive materials. Recently, the result shows octacalcium phosphate as osteoinductive which can develop immanent osteoinducitivity which it will open the restoration therapy. Furthermore, the quality is immanent for example developing without artificial introduction of osteogenic or growth factors (Barinov & Komlev, 2010). Report had state that OCP enhanced bone formation more than the stoichiometric HAp when they are implanted in the murine bone. (Suzuki *et al.*, 2006)

In addition, OCP have been implicated as possible precursors to the formation of apatite. This scenario may occur by the initial precipitation of OCP which followed by transformation to more formation of apatitic phases. In the biological apatite, OCP always found only during pathological calcification where the pH relatively low (Johnsson and Nancollas, 1992). At a controlled ionic strength of 0.10 mole/liter and with Ca/P ratio 1.33, the pH for the precipitation OCP are seem to be limited in range 6-7 where the precipitation of OCP at low supersaturation. (De Rooij et al. 1984).

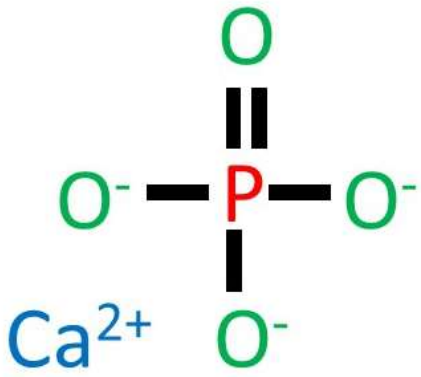

Figure 2.5: Chemical structure of Octacalcium phosphate (OCP) (LeGeros, 1985)

2.3.4 Hydroxyapatite (HAp)

Hydroxyapatite (HAp) is a material that compatible with living cells and allows new bone formation and adhesion. The chemical structure if hydroxyapatite is $Ca_{10}(PO_4)_6(OH)_2$. Figure 2.6 shows the structure of the hydroxyapatite (HAp). Moreover, hydroxyapatite is recognized as the golden material bone as well as for teeth also. This because it has similar properties to inorganic part of the bone (Dapporto *et al.*, 2016).

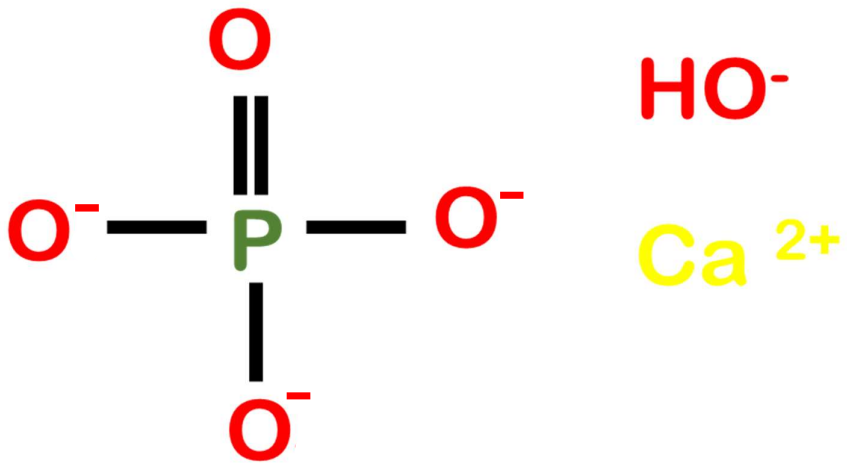

Figure 2.6: Chemical structure for hydroxyapatite (HAp) (Frasnelli and Vincenzo M Sglavo, 2016)

In addition, hydroxyapatite also categorized as family of apatite mineral. It has been widely used and studied by many researchers because it have the ability to formed through an acid-base reaction (Ginebra *et al.*, 2012). Moreover, hydroxyapatite is the most suitable ceramics material for bone regeneration because it has good biocompatibility. Furthermore, hydroxyapatite is chemically match with element of hard tissue. For example, calcium and phosphorus. Component of element structure in living

body such as bone and teeth likely to have a same structure with artificial hydroxyapatite as presented in Figure 2.7 (Sopyan *et al.* 2007).

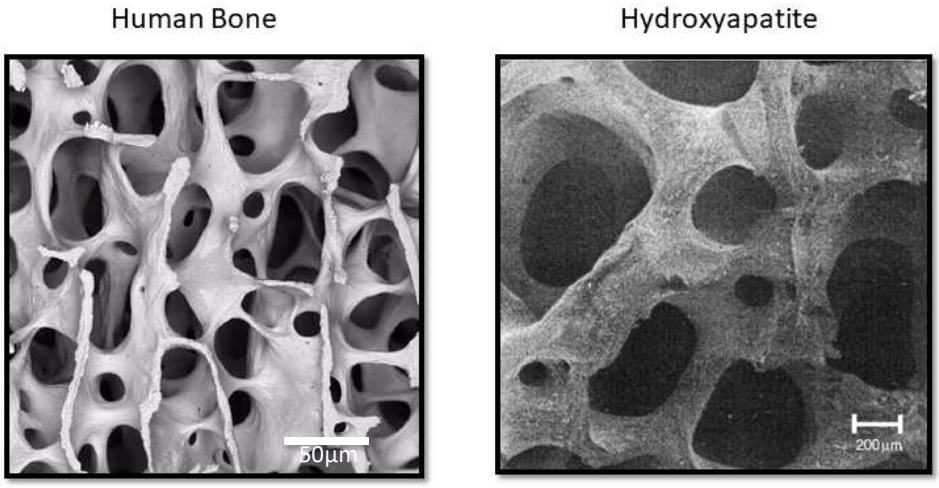

Figure 2.7: Comparison between human bone and artificial bone hydroxyapatite (Sopyan *et al.*, 2007)

Moreover, hydroxyapatite possess good biological compatibility and able to stimulate growth of immature cells. Hence, due to its excellent compatibility properties, hydroxyapatite are used in bulk form, coating and cement (Yang *et al.*, 2014). As the result it able to stimulate growth of immature cell and biologically active within living cells. Next, hydroxyapatite are extensively been used as bone substitutes in orthopedics and also in dental applications (Al-Sanabani *et al*. 2013).

Although hydroxyapatite have a variety of advantages, it also has some disadvantages. The disadvantages of hydroxyapatite are it possess low strength when it applied by mechanical force and low toughness in an application of load-bearing are applied (Kong *et al.*, 1999).

2.3.5 Tricalcium phosphate (TCP)

The tricalcium phosphate (TCP) have chemical compostion $Ca_3(PO_4)_2$ which is classified as a typical bioresorbable. It popular as scaffold materials for bone regeneration (Kamitakahara *et al*, 2008). They found that it can be used as bone substitutes, metallic prosthesis coatings, cements, and composite materials. A few sorts of tricalcium phosphates are used as biomaterials. In addition, high-temperature will forms α-TCP and β-TCP, an amorphous tricalcium phosphate and apatitic tricalcium phosphate which assume is a vital part in metallic prosthesis coatings and in calcium phosphate cements.(Rey *et al.*, 2008).

2.3.5.1. α-Tricalcium phosphate (α-TCP)

α-TCP is considered to be an excellent material used for injectable cement, degradable ceramic and bone regeneration component. *(*Frasnelli *et al.* 2016). Normally, α-TCP formed through sintering at temperature above than 1150°C. If the temperature is below than 1150°C, formation of β-TCP would be occured. α-TCP is a well-known for its excellent solubility. In fact, α-TCP shows the highest solubility among other materials in calcium phosphate groups (Carrodeguas & De Aza, 2011). α-TCP mainly used as fine powder to prepare calcium phosphate cement due to its good solubility and reactivity. Generally, α-TCP had been used as materials for self-setting bone cement (Carrodeguas & De Aza, 2011).

2.3.5.2. β-Tricalcium phosphate (β-TCP)

β-TCP is stable at low temperature (1100 °C) which is at the range of 1100 °C – 1150 °C. The β-TCP structure as shown in Figure 2.8 (Frasnelli *et al.* 2016). β-TCP phosphate are having same chemical composition as α-TCP but there are certain different in density and level of solubility (Frasnelli *et al.* 2016). Different in bio compatibility properties also determine the different in biological compatibility and medical application.

β-TCP comes in numerous kind of types which are scaffolds, cements and granules. Normally, it had been applied as an artificial bone substitute is β-TCP granules since bone imperfection or defect can be reproduced by essentially filling it with granules. But the downside is β-TCP granules frequently stream out from the defect area. Previous studies, they were using high purity of β-TCP ceramic granules to fill defect as shown in Figure 2.9 (Alan *et al.*, 2016).

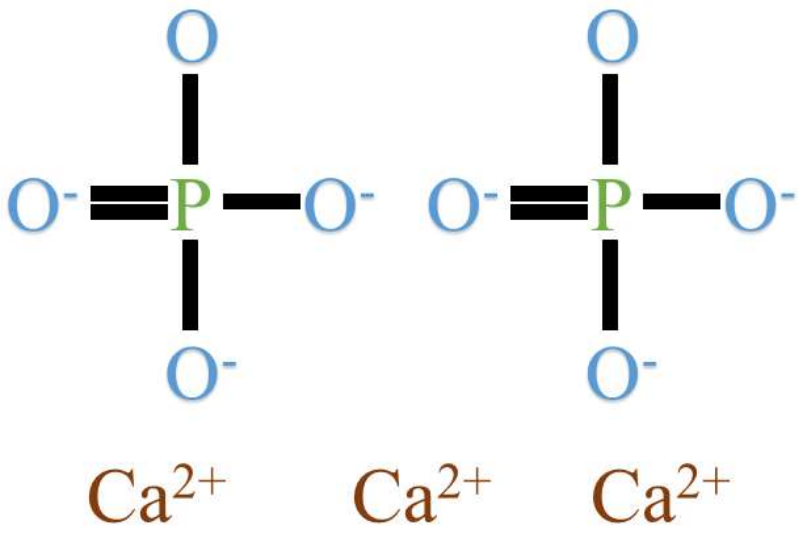

Figure 2.8: Structure of β-tricalcium phosphate (Frasnelli *et al,* 2016*)*

β-TCP is good as bone implantation due to its biological compatibility, bio-safe, high level of hygiene and also having a long life span. In addition, β-TCP shows a good balance among absorption, degradation and new bone formation (Frasnelli *et al*, 2016).

In Figure 2.10, the osteogenesis was observed between the granules and immature bone specifically joined to the porous structure. Through this finding, β-TCP is an appropriate material for the filling of bone deformities in light of its versatility, low complication rate, and ideal long-term result. The absence of remote body reaction or toxicity supports the usefulness of implant as fit substitute bone transplant to repair defect.

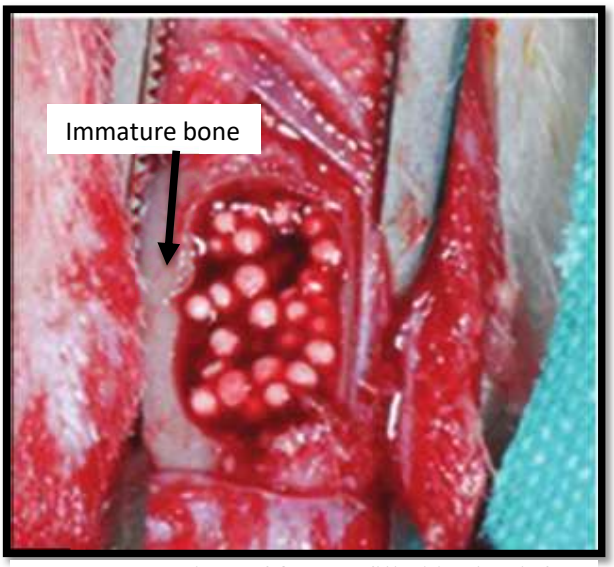

Figure 2.9: View of β-TCP filled in the defect (Alan *et al.*, 2016).

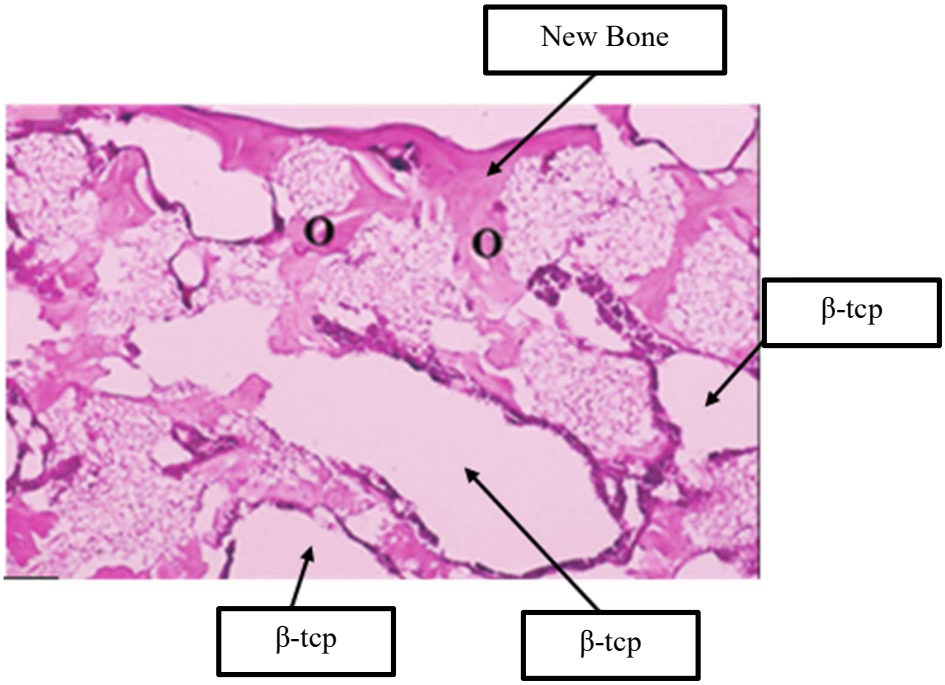

Figure 2.10: The photomicrograph shows growth of living tissues by β-tricalcium phosphate (Alan *et al.*, 2016)

2.4 β-tricalcium phosphate for clinical applications.

2.4.1 β-tricalcium phosphate scaffolds

Bone tissue engineering has been known as a good approach for repairing and reconstruction of bone. The scaffold is one of the key components of bone tissue development before it gradually degrading and after it is replaced by the new tissue. For regeneration of vast and complex bone tissue, the scaffold must have several structures features such as good control of scaffold porosity and architectural parameters such as pore geometry, size, interconnectivity, orientation and branching. Those are important to maximize nutrient diffusion and interstitial fluid stream to control cell development and function (Maté-Sánchez De Val *et al.*, 2014).

Moreover, scaffold must present with interconnected porous structure that have more than 100-200 μm of pore size. This range of pore size are the most proper size that would allow and empowering cell infiltration. Other than that, mechanical quality of scaffold should be good enough so that scaffold able to sustain mechanical stability when cell attached to form a new cell around the graft (Yang *et al.*, 2014).

2.4.2 β-tricalcium phosphate granules cements

β-tricalcium phosphate cement generally produced by mixing of calcium phosphate groups. By mixing it with aqueous solution it will then form a phase that ready to set and solidify consequently to form a cement. The cements are able to set and solidify through process of dissolution and precipitation. (Fukuda *et al.*, 2018)

In figure 2.11 β-TCP will form brushite as final product through acid base reaction by adding acid such as monocalcium phosphate monohydrate (MCPM) as shown in Figure 2.11(Ginebra *et al.* 2012).

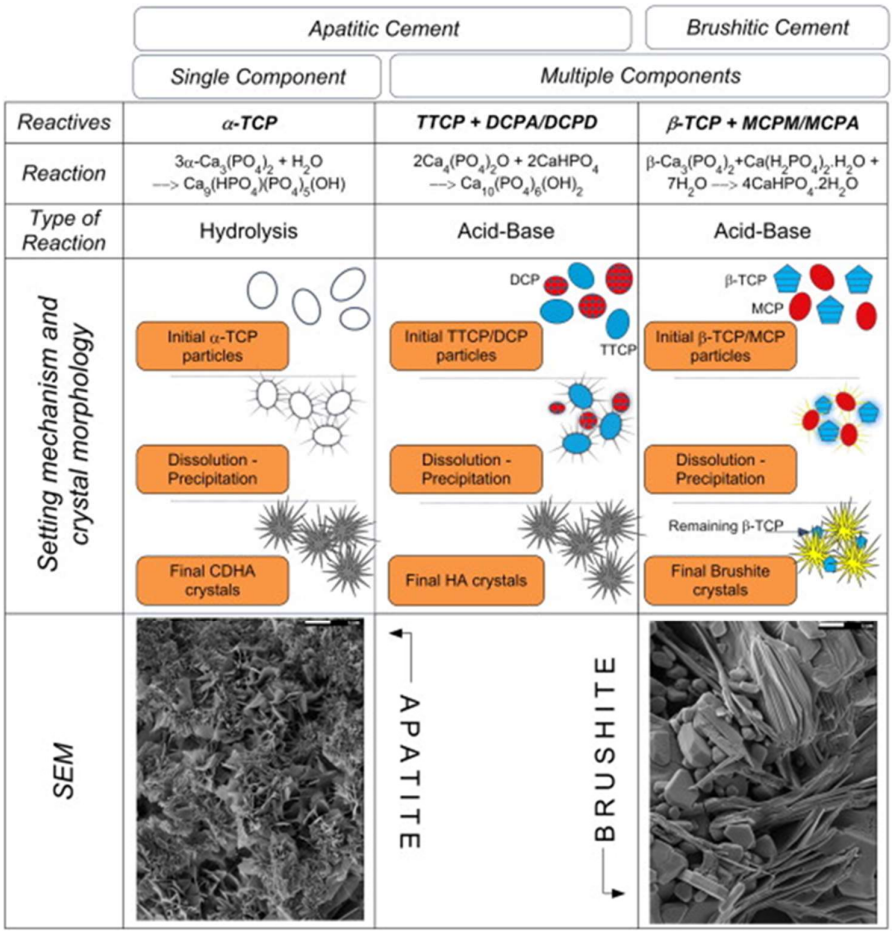

Figure 2.11: Classification of calcium phosphate cement by Ginebra *et al.* (2012).

Figure 2.11 shows the example of the normal formulations that classified type of phosphate cements. The cement was categorized by their final product which are either HAp or DCPD. Although, the number of component in the solid phase either single or multiple, hydrolysis or acid-base setting reaction, the setting mechanism and microstructure evolution still happen during setting (Ginebra *et al.* 2012).

In order to form β-TCP cement, there are three main setting stages need to be through. As described by Chen *et al*, (2002), the first stage is dissolution of β-TCP then followed by growing the new crystal and final step is crystalline arrangement. In other word, the setting reaction basically done through dissolution–precipitation process. 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. Second stage is nucleation of new phase. Nucleation will occur when Ca^{2+} and PO_4^{3-} ions reaches a supersaturated level. Nucleation formation will take place surrounding powder particles. At the final stage, the crystal phase will form if acidic solution still undergoes dissolution reaction. At initial the reaction occurs between the reagent and starting material. However, when crystalline phase starts to occur by precipitation of crystal.

Usually, β-TCP are formed as porous structure. The porosity will appear along the gap between precipitated crystals. The presences of pore sizes are in the micrometer size (Espanol *et al.*, 2009). The porosity need to be considered in formation of β-TCP cement because the porosity effect to enhance materials bioactivity and resorbability. Based on the porosity in structure, the exposure of large surface area for cell to attach are occur. Proper amount of porosity will allow materials to lead some good carrier properties to the cell at the same time it will improve bone remodeling process.

In addition, all these properties will diverge due to the processing settings which are restricted for solution to solid ratio and size of particles as shown in Figure 2.12. If liquid to powder ratio arise, the total porosity will also rised. Moreover, the coarser the powder it will increase the pore distribution which suitable for the application in clinics

and hospitals. Other than that, size and shape particle of starting materials can affected porosity (Ginebra *et al.*, 2012).

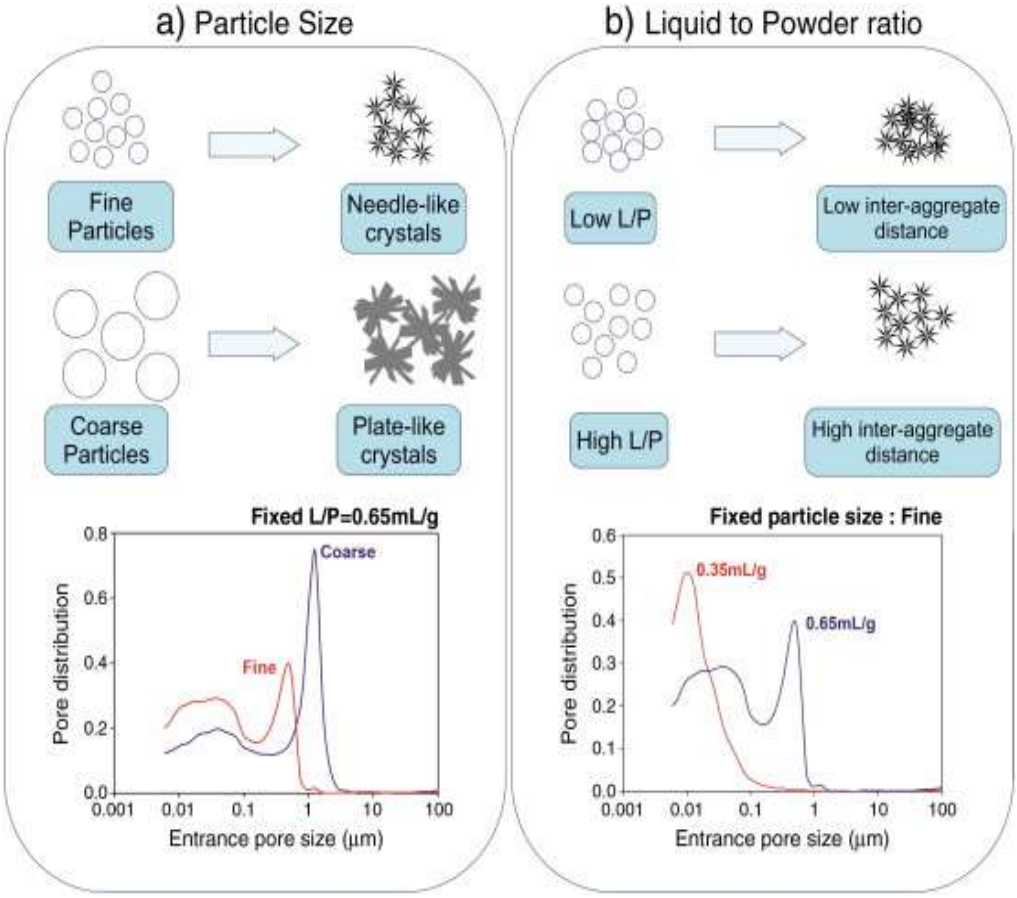

Figure 2.12: Condition of cement with varies (a) particle size and (b) L/P ratio (Ginebra *et al.* 2012)

Apart from that, calcium phosphate also has its bioactivity properties. It is because, in bone substitution, bioactivity is to assist materials so that it will strongly bind directly with the surrounding bone without have reaction to the other living cell around such as macrophages or might be osteoclast. Moreover, bioactivity properties purpose to avoid chemical dissolution by inactive resorption (Ginebra *et al.* 2012).