PRELIMINARY STUDY OF DICALCIUM PHOSPHATE DIHYDRATE COATED 45S5 BIOGLASS GRANULE

GAN SOON XIN

UNIVERSITI SAINS MALAYSIA

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SCHOOL OF MATERIALS AND MINERAL RESOURCES ENGINEERING

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PRELIMINARY STUDY OF DICALCIUM PHOSPHATE DIHYDRATE COATED 45S5 BIOGLASS GRANULE

By

GAN SOON XIN

Supervisor:

Dr. Khairul Anuar Shariff

Co-Supervisor:

Assoc. Prof. Ts. Ir. Dr. Hasmaliza Mohamad

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DECLARATION

I hereby declare that I have conducted, completed the research work and written the dissertation entitled **'Preliminary Study of Dicalcium Phosphate Dihydrate Coated 45S5 Bioglass Granule'**. I also declare that it has not been previously submitted for the award of any degree and diploma or other similar title of this for any other examining body or University.

Name of Student: **GAN SOON XIN** Signature:

Date: 12 August 2022

Witness by

Supervisor: **Dr. Khairul Anuar Shariff** Signature:

Date: 12 August 2022

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ACaP	Acidic Calcium Phosphate
Al_2O_3	Alumina
Ca	Calcium
$CaCO_3$	Calcium Carbonate
CaO	Calcium Oxide
CaP	Calcium Phosphate
Co	Cobalt
CoCr	Cobalt Chromium
DCPD	Dicalcium Phosphate Dihydrate
Fe	Iron
H	Hydrogen
H_3PO_4	Phosphoric Acid
HA	Hyaluronic Acid
HAp	Hydroxyapatite
HBSS	Hank's Balanced Salt Solution
MCPA	Monocalcium Phosphate Anhydrate
MCPM	Monocalcium Phosphate Monohydrate
Mg	Magnesium
MSc	Mesenchymal Cell
Na	Sodium
Na_2O	Sodium Oxide
$NaCO_3$	Sodium Carbonate
Ni	Nickel
OCP	Octacalcium Phosphate
P	Phosphate
P_2O_5	Phosphate (V) Oxide
PCL	Polycaprolactone
PDLLA	Poly (D,L-lactic acid)
PGA	Poly (glycolic acid)
PLA	Poly (lactic acid)
POP	Plaster of Paris
PPE	Polyphosphoester

PTMC	Polytrimethylene Carbonate
PUs	Polyurethanes
SEM	Scanning Electron Microscope
SiO_2	Silicon Dioxide
THP	Total Hip Replacement
Ti	Titanium
TTCMSs	Tetracycline-loaded Poly-β-hydroxybutyrate (P(3HB))
TTCP	Tetracalcium Phosphate
XRD	X-Ray Diffractometer
XRF	X-ray Fluorescence Spectroscopy
Zn	Zinc
ZrO_2	Zirconia
α-TCP	α - Tricalcium Phosphate
β-TCP	β - Tricalcium Phosphate

LIST OF SYMBOLS

º	Degree
ºC	Degree Celsius
g	Gram
L	Litre
mg	Milligram
ml	Millilitre
mm	Millimetre
mmol/L	Millimoles per Litre
mol%	Mole Percentage
rpm	Revolutions per Minute
wt%	Weight Percentage
µm	Micrometre

ABSTRAK

Dalam kajian ini, dikalsium fosfat dihidrat (DCPD) yang mempunyai keterlarutan yang tinggi akan disalut pada permukaan 45S5 biokaca untuk meningkatkan kadar pertumbuhan tulang. Objektif kajian awal ini adalah untuk mengkaji kesan kepekatan larutan asid kalsium fosfat terhadap pembentukan salutan dikalsium fosfat dihidrat (DCPD) pada permukaan granul 45S5 biokaca dan kesan tempoh pendedahan dalam fabrikasi 45S5 biokaca dengan salutan DCPD. Pertama sekali, granul 45S5 biokaca akan dihasilkan dengan kaedah cair-pelindapkejutan dan 45S5 biogelas yang terhasil akan disahkan melalui permerhatian morfologi, analisis unsur dan analisis komposisi. Bagi mencapai objektif pertama, granul 45S5 biokaca akan didedahkan dalam larutan asid kalsium fosfat yang berbeza kepekatan (25 mmol/L, 50 mmol/L, 75 mmo/L, 100 mmol/L) selama 2 jam untuk membentuk salutan DCPD pada permukaan granul 45S5 biokaca melalui tindak balas pelepasan-penghasilan. Manakala bagi objektif kedua, kepekatan bagi asid kalsium fosfat ditetapkan pada 100 mmol/L dan granul 45S5 biokaca direndam dalam larutan selama 2 jam dan 6 jam. Beberapa kaedah pencirian seperti Mikroskop Elektron Imbasan (SEM), Pendarfluor X-Ray (XRF), Pembelauan Sinar-X (XRD) dan analisis pH telah dijalankan terhadap granul 45S5 biokaca dengan salutan DCPD. Hasil analisis menunjukkan bahawa jumlah kristal DCPD yang terbentuk pada permukaan granul 45S5 biokaca meningkat apabila kepekatan larutan asid kalsium fosfat dan tempoh pendedahan ditingkatkan. Dalam kajian awal ini, walaupun DCPD dikesan terbentuk pada permukaan granul 45S5 biokaca, analisis pada granul menunjukkan bahawa DCPD tidak berjaya meliputi sepenuhnya permukaan granul 45S5 biokaca dan oleh itu kajian lanjut diperlukan untuk mengkaji kaedah yang paling sesuai untuk membentuk salutan DCPD yang sempurna pada permukaan granul 45S5 biokaca.

ABSTRACT

In this study, dicalcium phosphate dihydrate (DCPD) which has higher solubility is being coated on the 45S5 bioglass granule surface to improve the bone regeneration rate. The objectives of this preliminary study are to determine the effect of acidic calcium phosphate solution concentration in fabricating dicalcium phosphate diphosphate (DCPD) coated 45S5 bioglass granules and its effect of exposure duration when soaking in acidic calcium phosphate solution. 45S5 bioglass is first fabricated by using melt-quench method and the fabricated 45S5 bioglass is verified through morphology observation, elemental analysis and compositional analysis. To achieve the first objective, 45S5 bioglass granules was exposed to acidic calcium phosphate solution with different concentration (25 mmol/L, 50 mmol/L, 75 mmol/L, 100 mmol/L) for 2 hours to form a DCPD layer on the 45S5 bioglass granule surface through dissolution-precipitation reaction. While for the second objectives, the concentration of acidic calcium phosphate was fixed at 100mmol/L and 45S5 bioglass granules were soaked in the solution for 2 and 6 hours, respectively. The DCPD-coated 45S5 bioglass are then characterized with Scanning Electron Microscope (SEM), X-Ray Fluorescence Spectroscopy (XRF), X-ray Diffractometer (XRD) and pH analysis. The results showed that the amount of DCPD crystals precipitate on the 45S5 bioglass granule surface increased as the concentration of ACaP solution and exposed duration increased. In this study, even the DCPD is detected to be precipitated on the 45S5 bioglass granule surface, the results has shown that the DCPD precipitated is not fully covered the 45S5 bioglass granule surface and further study is required to understand the appropriate method to fully cover the 45S5 bioglass granules surface with DCPD.

CHAPTER 1

INTRODUCTION

1.1 Background of Research

Nowadays, biomaterials such as biometal, biopolymer and bioceramic are widely used in biomedical application and being implanted in human body. Compared to biometal and biopolymer, bioceramic which has similar composition of the original host bone allow it widely used to fabricate bone graft for bone regeneration application. To be used as an implant, bioceramic must have certain criteria which including biocompatible, good host response, non-toxic and good mechanical properties. Among three categories of bioceramic (bioinert ceramic, bioactive ceramic and bioresorbable ceramic), bioactive ceramic which able to make contact with the living bone and repair bone tissue are gain more attention nowadays. 45S5 bioglass which classified as bioactive ceramic is the first bioactive ceramic that was created by Hench LL in 1971 (Hench, 2006). 45S5 bioglass is a quaternary system with 45 wt% of silica (SiO_2), 24.5 wt% of calcium oxide (CaO), 24.5 wt% of sodium oxide (Na_2O) and 6.0 wt% of phosphorus pentoxide (P_2O_5). The calcium to phosphorus ratio of 45S5 bioglass is approximately equal to 5. 45S5 bioglass, a Class A bioactivity material which able leads to osteoconduction and osteostimulation of new bone has been widely used in clinical practises. However, high percentage of SiO_2 which act as network forming will strongly decreases the solubility rate of the other ions and hence cause the bone regeneration rate to become slow. It was reported that the calcium and phosphate ion released in the bone defect area will improved the bone remodelling rate of the bone substitute made from bioceramic materials. Therefore, since 45S5 bioglass contained high percentage of SiO_2 which reduce its biodegradation rate, a preliminary study to coat 45S5 bioglass surface with dicalcium phosphate dihydrate (DCPD) which has higher solubility will be embarked to see whether this technique is

feasible or not to improve the biodegradation rate of the 45S5 bioglass. Since calcium and phosphate ion released is crucial in stimulating bone remodelling process, therefore, in this study, effect of acidic calcium phosphate solution concentration and exposure duration in fabricating DCPD-coated 45S5 bioglass granule will be elucidated.

1.2 Problem Statement

45S5 bioglass had widely used as bone substitute materials since it has ability to form strong bond with soft tissue which helps in stimulating bone remodelling process. However, 45S5 bioglass tend to produce low biodegradation rate after implanted in bone defect area which decreased efficiency to form a new bone. Till date, there are many studies had been carried out to improve the biodegradation rate of 45S5 bioglass. For example, Hoppe *et al.* (2021) reported that the biodegradation rate of 45S5 bioglass were improved after incorporate copper into 45S5 bioglass composition (Hoppe *et al*., 2013). Meanwhile, Lopes *et al.* (2020) reported that incorporating niobium into the 45S5 bioglass formulation increased the released rate of calcium and phosphate ions after implanted in the bone defect area (Lopes *et al.*, 2020). Zhang *et al.* (2021) reported that bone-regeneration rate of 45S5 bioglass is enhanced by inducing strontium in the 45S5 bioglass formulation (Zhang *et al.*, 2021). Besides copper, niobium and strontium, Joy-anne *et al.* (2021) reported that the incorporation of boron oxide and gold into the 45S5 bioglass helped in improving the bone remodelling rate of 45S5 bioglass (Joy-anne *et al.*, 2021). Although this doping method which involve element such as copper, niobium, strontium, boron oxide and gold successfully improved the biodegradation rate of 45S5 bioglass, but this method is complex in addition high cost. Therefore, simple fabrication process and cheaper cost should be proposed in order to broaden its clinical usage and applications.

In this study, preliminary study to coat 45S5 bioglass with dicalcium phosphate dihydrate (DCPD) through dissolution-precipitation process will be elucidated. Previous studies had reported that the DCPD shows high solubility and good osteoconductivity at physiological body condition (Khairul *et al*., 2017; Han *et al*., 2021). Therefore, it was expected that by introducing DCPD coating layer on 45S5 bioglass granular surface, the biodegradation rate of the bioglass will be increased and released more Ca and P ions that helps in stimulating cell activities which induce fast new bone formation. It was expected that the preliminary results obtained in this study will give a new insight to all bioceramic researchers' regarding the feasible method to coat DCPD layer on the 45S5 bioglass surface.

1.3 Research Objectives

The objectives of this project are

1. To determine the effect of acidic calcium phosphate solution concentration in fabricating DCPD-coated 45S5 bioglass granules.
2. To investigate the effect of exposure duration in fabricating DCPD-coated 45S5 bioglass granules

1.4 Research Approach

For this project, DCPD-coated 45S5 bioglass granule were prepared using the dissolution-precipitation method with different concentration of acidic calcium phosphate (ACaP) solution and different exposure duration. 45S5 bioglass which is the primary material was first fabricated using melt-quench method. During the fabrication of 45S5 bioglass, silicon dioxide (SiO_2) powder, calcium carbonate ($CaCO_3$) powder, phosphorus pentoxide (P_2O_5) powder and sodium carbonate (Na_2CO_3) powder was weighed

according to the weight ratio of 45S5 bioglass and mixed for 6 hours using roller mixer with speed of 35 rpm. The mixed powder is then melted at 1400 ºC with soaking time of one hour. The melted mixture is then quenched in distilled water and bioglass frit can be obtained. The bioglass frit is then dried in oven overnight and milled into powder form. The 45S5 bioglass powder was pressed into pellet form with a hydraulic hand press machine and these pellets is then crushed to obtain 300 μm-600 μm granular size.

To coat the 45S5 bioglass with DCPD, acidic calcium phosphate (ACaP) solution will be prepared first. To prepare different concentration of ACaP solution, different amount of monocalcium phosphate monohydrate (MCPM) powder were dissolved in 25 mmol/L of phosphoric acid. The mixture is shaked to dissolve the MCPM powder in the phosphoric acid. During the coating process, 45S5 bioglass were exposed to ACaP solution in a bottle with the liquid to granular ratio of 1.5 ml:0.2 g. The bottles were then placed on the overhead shaker and shaked at a speed of 125 rpm. After soaking for 2 hours, the specimens were rinsed with acetone to stop the reaction and the specimen were dried in oven for 1 hour with the temperature of 60 ºC. The fabricated specimen is then characterized using Scanning Electron Microscope (SEM), X-Ray Fluorescence Spectroscopy (XRF) and X-ray Diffractometer (XRD). Besides that, the bioactivity properties of the specimen was being analyzed also by soaking the specimen in the Hank's balanced salt solution (HBSS) and the pH analysis was carried out to determine the pH value of HBSS after immersion for 1,3,5, and 7 days. Therefore, the procedure to fabricate the DCPD-coated 45S5 bioglass granule and the characterization of the specimen were shown in Figure 1.1 below.

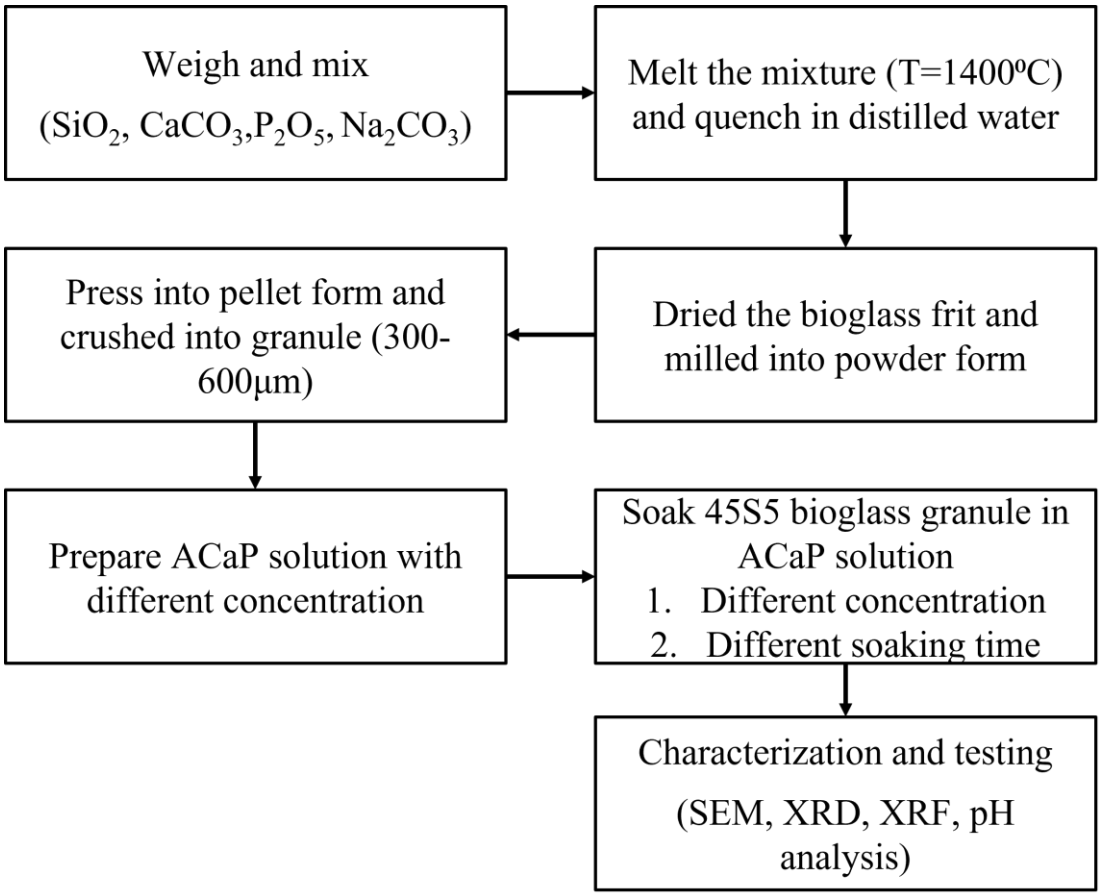

Figure 1.1: Fabrication and characterization steps for DCPD-coated 45S5 bioglass granules

CHAPTER 2

LITERATURE REVIEW

2.1 Biomaterials for Bone Regeneration Application in Tissue Engineering

Biomaterials is a synthetic or natural materials that being used as replacement for any defect that may exist inside a mammal or human due to genetic defects, age, illness, trauma, or injuries. Figure 2.1 below has shown the interdisciplinary system of biomaterials. During the fabrication process of a biomaterials, there are some knowledge and ideas required which are materials science and engineering, chemistry, medical science, biophysics, biology and molecular biology to make sure the biomaterials can achieve the desired requirement as a replacement material (Kiran & Ramakrishna, 2021).

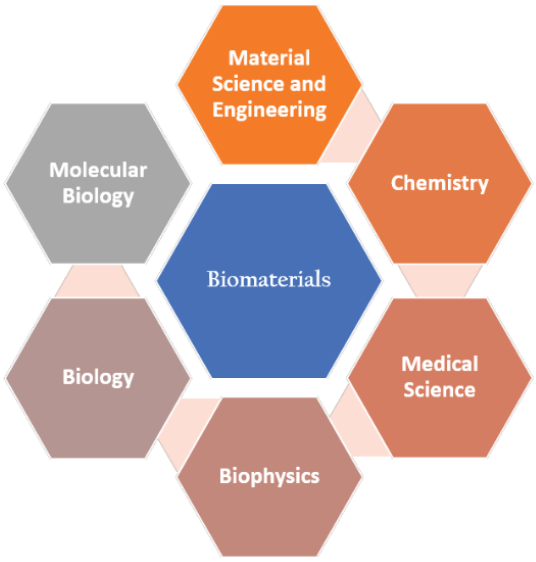

Figure 2.1: Interdisciplinary system of biomaterials (Kiran & Ramakrishna, 2021)

To achieve the desired requirement as a biomaterial, some properties should be considered which are biocompatibility, host response, toxicity, mechanical properties, corrosion, wear and fatigue properties, design and manufacturability. Table 2.1 below summarized the properties that should considered on the biomaterials.

Table 2.1: Considered properties of Biomaterials (Kiran & Ramakrishna, 2021)

Properties	Explanation
Biocompatibility	• Ability of biomaterials to support cell growth without eliciting an inflammatory or immunogenic response
Host Response	• Response of the living body organism to the biomaterial
Non-toxicity	• The biomaterials which implant in living body should not release any toxic and not influencing the living body system
Mechanical Properties	• Properties such as tensile strength, yield strength, hardness and creep should be investigated before implantation
Corrosion, wear and fatigue properties	• Corrosion, wear and fatigue properties of the materials which may cause the biomaterials failed should be studied carefully before implantation
Design and manufacturability	• The design for the implant component should be customized according to the condition of patient and the manufacturability. • Manufacturability is the ability to fabricate the component with relative ease.

With the increasing of living quality, biomaterials have grown to become an integral component nowadays. The application of biomaterials has broadened from diagnostics and medical equipment to therapeutic medications and emerging regenerative drugs and these applications are widely used to address different health-related issue in body system such as skeletal system, cardiovascular system, organs and ophthalmologic. Table 2.2 below present the application of biomaterials in human body (Ratner *et al.,* 2020).

Table 2.2: Application of Biomaterials in Human Body (Ratner *et al*., 2020)

Body System	**Example of Application**
Skeletal System	Joint replacement, Trauma fixation device, Spine disks and fusion hardware, Bone cement, Bone defect repair, Cartilage, tendon, or ligament repair, Dental implant
Cardiovascular System	Vascular grafts, patches, and endovascular devices, Heart valve, Pacemakers, Implantable defibrillators, Stents: Coronary, Peripheral, vasculature, Nonvascular, Catheters: Cardiovascular, Urologic
Organs	Cardiac assist device, Hemodialysis, Blood oxygenator, Skin substitute
Ophthalmologic	Contact lens, Intraocular lens, Glaucoma drains
Other	Cochlear prostheses, Breast implants, Hernia and body wall repair meshes, Sutures, Blood bags, Ear tubes, Intrauterine device

In human body, bone is a connective tissue which has excellent bone regeneration ability to repair itself when there is any injury or defect. This ability is due to the removal of orchestrated of bone by osteoclasts and followed by formation of new bone by osteoblasts (Murphy *et al*., 2013). Osteoclasts, osteoblasts, and osteocytes and bone lining cells are the active cells which exist in mineralized bone matrix. Osteoblasts, bone lining cells and osteoclasts are the cells where present on the bone surfaces and these cells are derived from local mesenchymal cells (MSc) called progenitor cells while osteocytes present inside the bone and are produced from the fusion of mononuclear blood-borne precursor cells (Mohamed, 2008).

However, if the defect appeared is exceed a critical size (9 mm), the bone self-regeneration ability is insufficient to repair the defect and hence a surgical intervention is required. There are many therapeutic strategies can be applied to promoting bone tissue regeneration and one of the strategies is transplant of a bone graft. With the transplant of

bone graft in the bone defect area, bone healing and new bone regeneration will occur through the process of osteogenesis, osteoinduction, osteoconduction and osseointegration (Shrivats *et al*., 2013).

Osteogenesis is a process where the osteoblasts express the osteoid that subsequently mineralizes and, hence yielding new bone. Osteoinduction is an important property for bone regeneration since it will enable the bone graft to induce the formation of the bone-forming cells through the differentiation process of multipotent mesenchymal stem cells (MSCs) and hence produce osteoprogenitor cells followed by development of osteoblasts. Besides that, osteoconduction is the property where the bone graft supports the attachment of new osteoblasts and osteoprogenitor cells. Osseointegration can be defined as the ability to bind to the surrounding bone without an intervening layer of fibrous tissue and hence enable the incorporation of the graft at the host site (Polo-Corrales *et al*., 2014).

Bone tissue is a special tissue in the human body where it is build up by two different components which is cortical bone and cancellous bone. Cortical bone which also called as compact bone is hard and denser outer surface that surround the inner cancellous bone. This cortical bone also has higher mineral content and also higher strength compared to cancellous bone. While cancellous bone which also called as spongy bone or trabecular bone is found inside by the bone and it is surrounded by the cortical bone (Battafarano *et al*., 2021). Figure 2.2 below has show the cortical bone and cancellous bone.

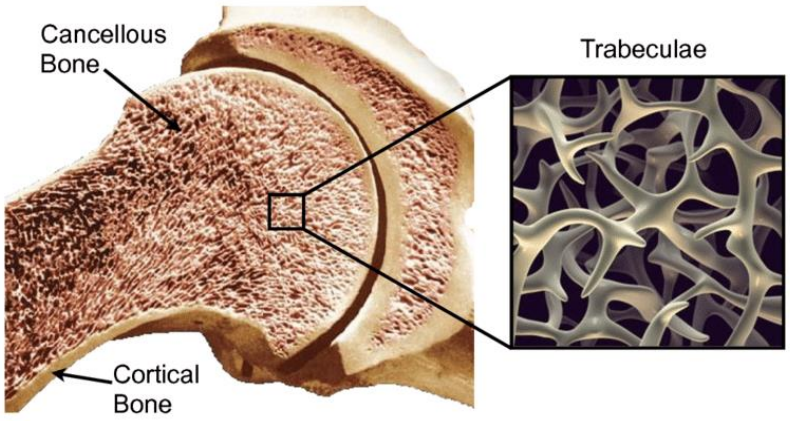

Figure 2.2: Cortical bone and cancellous bone (Cardiff, 2012)

Hence, for different application, the bone graft used may be cortical, cancellous or cortico-cancellous. Cortical bone graft is used for structural support and this bone graft may be used as onlay graft which mostly used in dental surgeon by graft it on the top of bone to support the upper jaw. Different with cortical bone graft, cancellous bone graft is used for osteogenesis and it normally used in fracture non-union, dental defects and other small bone defects since it is lack of mechanical strength but easier to use. Besides that, the porous structure of cancellous bone graft enable bone ingrowth and hence improve healing. Hence to combine both of structural support and osteogenesis, cortico-cancellous bone graft which can provide structural support and also provide osteogenesis for bone healing (Oryan *et al*., 2014).

For bone tissue regeneration, there are three main elements which are bone graft, cells and growth factors. For the bone graft, there are two option of bone graft available for bone healing which is natural bone graft or synthetic bone graft. While from these two options, natural bone graft can be classified to autograft, allograft and xenograft while synthetic bone graft can be classified to synthetic bone graft and bone regenerative medicine bone graft as shown in Figure 2.3 below.

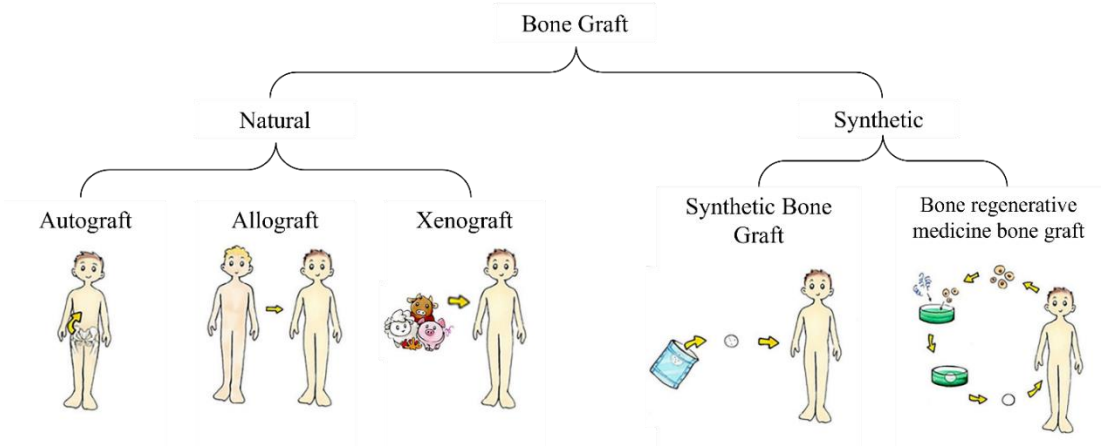

Figure 2.3: Classification of Bone Graft (Offner *et al*., 2019)

Autograft is a bone material that removed from body part of patient and then grafted into the bone defect area. Autograft are considered as gold standard for bond grafting application since it fulfills the bone regeneration criteria which are osteoconductive, osteoinductive and it was able to promote osteogenesis. Besides that, since the autograft is taken from patient's body, it will decrease the risk of immunological sequalae. Allograft is a bone material that removed from an individual of same species and bone grafted into bond defect area of patient after treatment such as decontamination. This allograft can avoid the patient to undergo second surgical. However, allograft may cause immune response in the new host. While for xenograft, it is a bone material which removed from other species and being grafted into bond defect area after the decontamination process. Xenograft also facing the same problem with allograft which may cause immune response. For the synthetic bone graft, it is a biocompatible bone graft that being fabricated by human. The synthetic bone graft can be used selectively according to the requirement in different case. However, this synthetic bone graft also has some limitation such as low mechanical strength, absorption rate and low ductility. Other than that, bone regenerative medicine bone graft is a product that cultured the patient's cell in laboratory on sterile media to stimulate the cell. The bone graft which consists of

the cell is then implanted into the bone defect area and this will increase the speed of bone regeneration (Offner *et al*., 2019).

2.2 Classification of Biomaterial for Synthetic Bone Graft

For synthetic bone graft, there are some materials can be used to fabricate the bone graft according to the application and requirement. This is because for different application in body system, there is some limitation for some materials to be used. The biomaterial that can be used to fabricate synthetic bone graft is polymer, metal and ceramic. Figure 2.4 below has shown the classification of biomaterials used for synthetic bone graft.

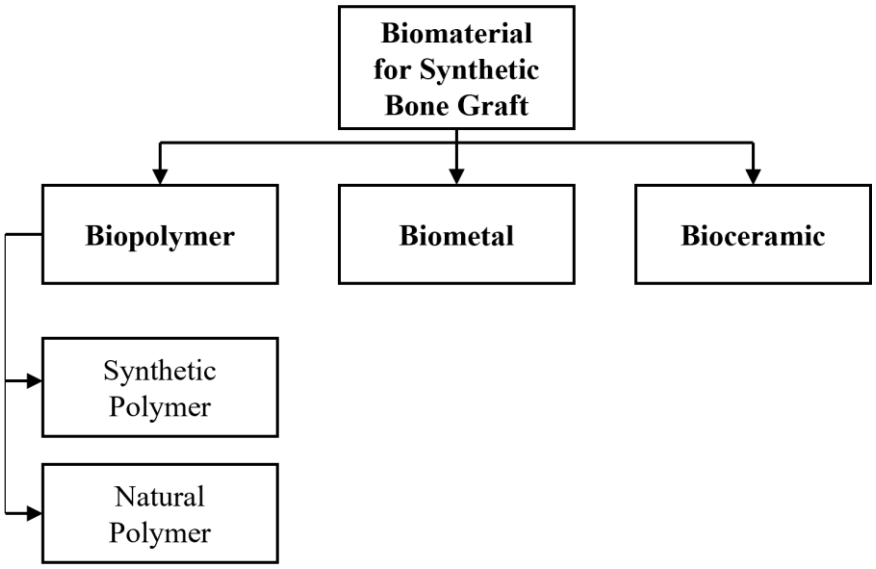

Figure 2.4: Classification of Bone Graft Material (Turnbell *et al*., 2018)

From Figure 2.4, it has shown that the general materials used that can be used to fabricate synthetic bone graft are polymer, metal and ceramic. For polymeric biomaterials, it also can be classified into natural or synthetic. Table 2.3 below has shown the general comparison of bone graft material.

Table 2.3: Comparison of Bone Graft Material (Turnbull *et al*., 2018)

Bone Graft Material	**Advantages**	**Limitation**
Polymer	• Natural polymer has high biocompatibility and low toxicity • Synthetic polymers have better physical properties • Biodegradable • Contain biofunctional molecules on surface • Biodegradable	• Natural and synthetic polymer has poor mechanical properties • Natural polymer may contain impurities such as endotoxin • Most synthetic polymer are hydrophobic and lack of cell recognition sites
Metal	• Biocompatible • High strength • Good mechanical properties	• High modulus which able lead to stress shielding • Poor biodegradability • Secondary release of metal ions
Ceramic	• Osteoconductive and osteoinductive • allow integration with host tissue • Similar composition with the mineral of host bone • Can be delivered as granules, paste or in an injectable format	• Hard and brittle • May display inappropriate degradation

2.2.1 Biometal

Metal have been used as biomaterials for a few decades since the metal is always in an equilibrium with its ions and these metal ions are responsible for biochemical function which are important for bone tissue regeneration as they will affect the equilibrium between osteoblasts, osteoclasts and osteocytes. Besides that, metal which has good mechanical properties also can provide support during the implantation process. The metallic biomaterials can be classified as bioinert metal and biodegradable metal as shown in Figure 2.5 below (Glenske *et al*., 2018).

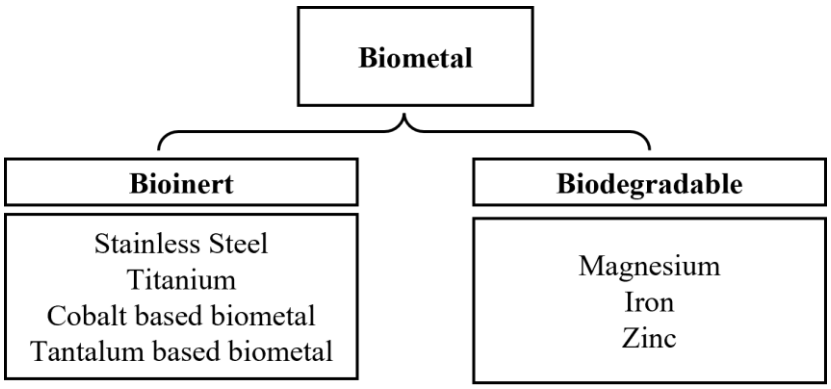

Figure 2.5: Classification of Biometal (Glenske *et al*., 2018)

Among bioinert metal, stainless less, cobalt-chromium (CoCr) alloys and titanium (Ti) alloys are mostly used as bone graft due to their high stability under reactive environment. Besides that, these metal which has excellent mechanical properties also suitable to be used as biomaterial. Stainless steel which used in biomedical field commonly classified as conventional stainless steels or Nickel free stainless steel which able to reduce the corrosion. The other bioinert metal is titanium and titanium-based alloys since the titanium dioxide layer that form on the surface of bone graft have higher dielectric constant and this will increase the cell integration process and strongly bond the Ti-based implant to the tissue. Other than that, cobalt (Co) based biometals also have higher wear resistance and this property allow it to be used in artificial hip joint. Last but

not least, tantalum-based biometal which have excellent corrosion resistance also allow it to widely use in biomaterials application. Besides that, the porous structure of tantalum also allow it has excellent bone-bonding properties which able to make the tantalum be an popular material to produce bone graft and even as coating on stainless steel and titanium to enhance the corrosive resistance properties (Prasad *et al*., 2017).

While for biodegradable biometals, magnesium, iron and zinc alloys are the existence alloy that able to provide vivo biocompatibility and also sufficient mechanical strength to support the bone. Magnesium which has similar properties as bone can reduce the risk of shielding after implantation. However, Mg which will rapid corrosion in physiological condition may lead to dissolve of Mg^{2+} ion and hence reduce the mechanical strength as a bone graft. Zinc (Zn) is another biodegradable biometal in biomedical field since it important for proper immune system functioning, cell division and for skeletal development. Besides from Mg and Zn, iron (Fe) which has lower corrosion rate will form a protective surface oxide layer that can prevent rapid degradation. Besides that, Fe also have high radial strength and hence allow for thin stent struts, produce ductile structure and make it more easier to deploy into the artery (Glenske *et al*., 2018).

2.2.2 Biopolymer

Among different biomaterials, polymeric biomaterials which has better biocompatibility and biodegradability are considered as the most promising candidates nowadays. Besides that, polymeric biomaterials can vary its properties according to desired requirement by manipulate their chemical composition and structure. The polymeric biomaterials can be classified according to the origin which are natural and synthetic as shown in Figure 2.6 below (Shi *et al*., 2016).

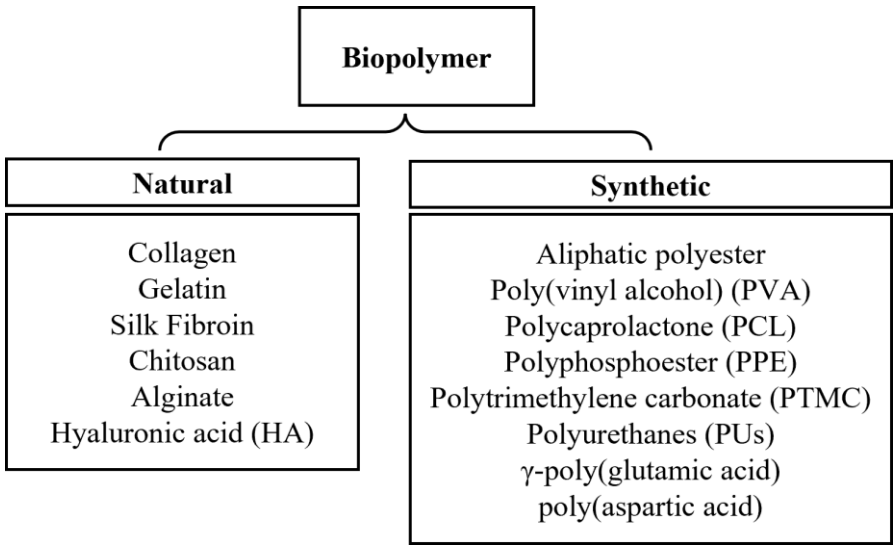

Figure 2.6: Classification of Biopolymer (Shi *et al*., 2016)

Among natural polymeric biomaterials, collagen is the major component in the natural bone tissue and type I collagen is the most suitable environment for osteogenesis since collagen is a non-toxic material and it is easy to obtain from different tissue. Gelatin which has higher solubility in water may be used to fabricate gelatin-based composite in order to increase the mechanical strength and osteoconductive performance. Silk fibroin is the structural protein of silk fibers which have high mechanical strength and thermal stability. The other natural polymeric biomaterials, chitosan is a positively charged polysaccharide where can interact with molecules with negatively charged. Chitosan is often combined with inorganic compound in order to improve its osteoconductive

properties. Different with chitosan, alginate is a polysaccharide with negatively charged and the mechanical and biological properties of alginate can be changed by vary the content of two monomers. Besides that, hyaluronic acid (HA) is an anionic glycosaminoglycan which have excellent viscoelasticity and water solubility (Shi *et al*., 2016).

Compared to natural polymeric biomaterials, synthetic polymeric biomaterials can be fabricated according to the required properties. Hence, the strength, degradation rate and microstructure of synthetic polymeric biomaterials more predictable and reproducible. Aliphatic polyester which consists of poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and copolymer (PLGA) are commonly used to fabricate bone graft. These polymers will degrade by non-enzymatic hydrolysis and will not produce toxic residue. Polycaprolactone (PCL) is also a biomaterial which have high crystallinity, good mechanical strength, slow degradation rate and poor water wettability. Polyphosphoester (PPE) is a polymer which consist of phosphate and have higher potential osteoinductivity for bone regeneration. Besides that, there also other polymeric materials that can be used in biomedical application such as polytrimethylene carbonate (PTMC), biodegradable polyurethanes (PUs), γ-poly(glutamic acid) and poly(aspartic acid) (Shi *et al*., 2016).

2.2.3 Bioceramic

Bioceramics is a class of materials that are used for repairing or replacing damaged bone tissue, and these materials have been widely used since the second half of the 20th century. Until nowadays, the evolution of bioceramic can be explained as three generations. For the first generation, bioinert and non-absorbable ceramics were used as the replacement of defect in human skeleton. In the second generation, biodegradable and bioactive ceramic materials which can dissolve in physiological environment after a certain period of time and tightly bond to living tissues were developed. With the improvement in technology, third generation bioceramics were developed and were used as a scaffold to support cells interactions during bone remodelling process. As summary, Table 2.4 below shows the tabulated information regarding the three generations of bioceramics used for bone repair. (Vallet-Reg, 2019).

Table 2.4: Three generation of bioceramic used for bone repair (Vallet-Regí, 2019)

	1st generation	2nd generation		3rd generation
Type of ceramic	Bioinert Non-absorbable	Biodegradable Resorbable	Bioactive	Scaffolds
In vivo reactivity	Isolated by a non- adherent fibrous capsule	Dissolved after a specific time	Tightly bonded to living tissues though	Stimulation living tissues regeneration
Example	• Alumina Al_2O_3 • Zirconia ZrO_2 • Carbons, • Sintered hydroxyapatite	• Calcium phosphate • Calcium sulfate	• Hydroxyapatite • Hydroxycarbonate apatite • Glasses • Glass ceramic	• Bioglass: particulate form • Porous scaffold bioactive and biodegradable ceramic

2.3 Bioresorbable Ceramic

Bioresorbable ceramic defined as a material that able to dissolve (resorbed) after implanted in human body starts and replaced by advancing tissue such as bone. The most widely used bioresorbable materials are tricalcium phosphate [$Ca_3(PO4)_2$] (Azom, 2004).

2.3.1 Calcium Phosphate

Calcium phosphates are compound which are composed of calcium (Ca) cation and phosphate (P) anion. Calcium phosphate compound are known as major inorganic materials in approximately 60% of all native human bones. Calcium phosphate compound has been widely used in bone regeneration application due to its osteoconductive and, in some cases, osteoinductive properties. Osteoinductive refers to the ability to induce progenitor cells to differentiate into osteoblastic lineages, while osteoconduction refers to the ability of bone growth on the surface of the materials (Albrektsson & Johansson, 2001). Osteoinductive and osteoconduction are important to ensure the bone graft able to support cell adhesion and proliferation. Cell adhesion is strongly influenced by the ability to adsorb extracellular matrix proteins and it is influenced by surface roughness, crystallinity, solubility, phase content, porosity, and surface energy. (Jeong *et al.*, 2019)

Meanwhile, calcium phosphate compounds can be categorised into different types such as α-tricalcium phosphate (α-TCP), hydroxyapatite (HAp), dicalcium phosphate dihydrate (DCPD), tetracalcium phosphate (TTCP), β-tricalcium phosphate (β-TCP) and monocalcium phosphate monohydrate (MCPM). Each of these compounds will have a different Ca/P ratio and solubility, and compounds that have a higher Ca/P ratio will have lower solubility. Table 2.5 shown the different type of calcium phosphate and its characteristics.

Table 2.5: Calcium Phosphate Compound (Kucko *et al*., 2019)

Calcium Phosphate Compound	**Abbreviation**	**Chemical Formula**	**Ca/P ratio**	**Solubility at 25ºC (mg/L)**
Monocalcium phosphate monohydrate	MCPM	$Ca(H_2PO_4)_2.H2O$	0.5	18,000
Dicalcium phosphate dihydrate	DCPD	$CaHPO_4.2H_2O$	1.0	88
Octacalcium phosphate	OCP	$Ca_8H_2(PO_4)_6.5H_2O$	1.33	8.1
β-Tricalcium phosphate	β-TCP	$Ca_3(PO_4)_2$	1.5	0.5
α-Tricalcium phosphate	α-TCP	α-$Ca_3(PO_4)_2$	1.5	2.5
Hydroxyapatite	HAP	$Ca_{10}(PO_4)_6(OH)_2$	1.67	0.3
Tetracalcium phosphate	TTCP	$Ca_4(PO_4)_2O$	2.0	0.7

Monocalcium phosphate monohydrate (MCPM) which has Ca/P ratio of 0.5 is the most acidic and water-soluble calcium phosphate compound. MCPM is fabricated through the precipitation process from highly acidic solution. At temperature of 100ºC, MCPM will transform into monocalcium phosphate anhydrate (MCPA) ($Ca(H_2PO_4)_2$). Since MCPM is highly acidic and high solubility, it cannot be used in biological calcification, and it commonly used to fabricate calcium phosphate cements (Dorozhkin & Epple, 2002).

Dicalcium phosphate dihydrate (DCPD) which also called as brushite is a biocompatible, biodegradable and osteoconductive bioceramic. DCPD which has a Ca/P ratio of 1.0 is highly soluble. DCPD can be fabricated by neutralized phosphoric acid with

calcium hydroxide in the condition of pH 3-4 and room temperature. From the neutralization process, DCPD can be obtained through the double decomposition process between calcium and phosphate containing solution in slightly acidic condition. Other than neutralization process, DCPD also can be fabricated by the conversion of calcium phosphates salts in acidic media and also by the reaction of calcium salts in acidic orthophosphate solutions (Tavoni *et al*., 2021).

Octacalcium phosphate (OCP) which has Ca/P ratio of 1.33 has higher solubility than tricalcium phosphate (TCP), hydroxyapatite (HAp) and tetracalcium phosphate (TTCP). OCP has been used as biomaterials since it has excellent bone regeneration properties since OCP able to promote response from the cells surrounding. It was found that OCP able to enhance the differentiation of osteoblasts and osteocytes and even formation of osteoclasts. These responses of OCP able to activate the bone regeneration and contribute to excellent biodegradability and osteoconductivity of OCP (Shiwaku & Suzuki, 2020).

Tricalcium phosphate (TCP) has two polymorphs which are high temperature α-Tricalcium phosphate (α-TCP) and low temperature β-Tricalcium phosphate (β-TCP). α-TCP has a monoclinic crystal structure while β-TCP has a rhombohedral crystal structure. β-TCP which formed at temperature of 900ºC - 1100ºC is more stable than α-TCP which formed at temperature of 1125ºC or higher. Since β-TCP is more stable at physiological body condition β-TCP is widely used for bone regeneration application. β-TCP also has higher high resorption rate and can be used to improve biocompatibility and promote the proliferation of osteoblasts and bone marrow stromal cell (Jeong *et al*., 2019).

Hydroxyapatite (HAp) is also one of the calcium phosphate compounds with the chemical formula of $Ca_{10}(PO_4)_6(OH)_2$. HAp is a naturally occurring form of calcium phosphate that exist in large amount in human bones. With the Ca/P ratio of 1.67, HAp

have lower solubility in physiological condition and hence HAp can be defined as a stable compound. HAp has been used for different application such as bone graft, injectable cements, coating for metallic implant and also drug delivery platforms. There are a few methods to fabricate HAp which including high temperature solid-state reaction and low temperature precipitation. HAp is osteoconductive but not osteoinductive and hence, substitution of ions such as fluoride, chloride and carbonate ions can improve the performance of HAp (Tavoni *et al*., 2021).

Tetracalcium phosphate (TTCP) with Ca/P ratio of 2.0 is less soluble compared to other calcium phosphate except hydroxyapatite. TTCP also is the most basic calcium phosphate compound and it cannot be precipitated by a solid-state reaction above 1300ºC. Besides that, TTCP is not very stable in aqueous solution. TTCP which have opposite properties with MCPM also not to be used in biological calcification but commonly used to prepare self-setting calcium phosphate cements (Dorozhkin & Epple, 2002).

2.3.2 Bioactive Properties of Calcium Phosphate

After the calcium phosphate compound being implant in human body, there will have reaction between the implant and human body. Figure 2.7 below show the example of phenomena that occur on the surface of hydroxyapatite after implantation.

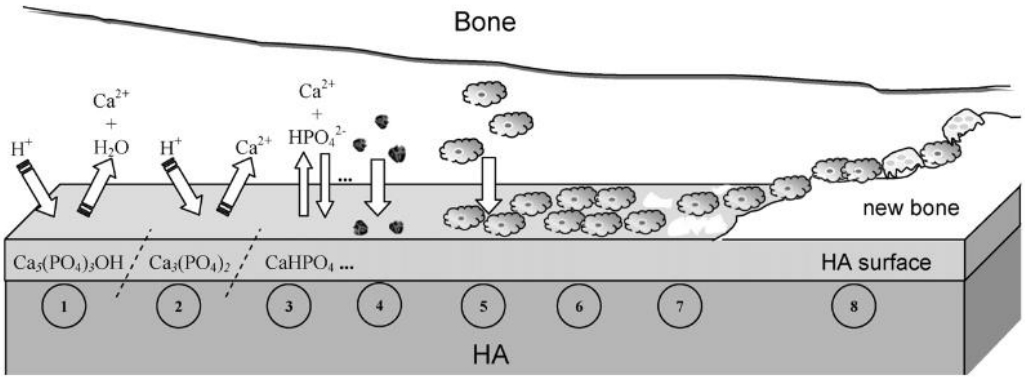

Figure 2.7: Phenomena that occur on the surface of hydroxyapatite after implantation. (Bertazzo *et al*., 2010)

From the Figure 2.7 above, the phenomena that occur after implantation can classified as eight stages where (1) solubilization of hydroxyapatite surface start, (2) continuation of solubilization hydroxyapatite surface, (3) Achievement of equilibrium between physiological solutions and modified surface of hydroxyapatite, (4) Adsorption of proteins and organic materials, (5) cell adhesion, (6) proliferation, (7) new bone production and (8) metabolism. (Bertazzo *et al*., 2010)

When the hydroxyapatite was implanted into body, the surface of hydroxyapatite will start to dissolve in the physiological solution until the equilibrium between the physiological solution and surface of hydroxyapatite is achieved and hence form the $CaHPO_4$ on the surface. With the equilibrium condition, protein from the body fluid will be absorb onto the surface of hydroxyapatite bone substitude. The ions on the surface of implant (Ca^{2+} and PO_4^{3-}) will bond to the charged functional group on the protein molecules. After the protein adsorption, the cells will attach to the surface mainly by an integrin receptor-mediated binding. Integrins are transmembrane heterodimeric glycoproteins with adhesion which able to bind to proteins and control all major cellular activities such as: adhesion, cell shape changes, proliferation, and migration. With the cell adhesion and proliferation, the new bone is started to regenerate. (Oliveira, Alves and Mano, 2014)

2.3.3 Calcium Phosphate as Coating Materials for Implants

Recent years, the implants that widely used commonly made from metal such as Magnesium (Mg), Titanium (Ti) and stainless steel. Even these metal implants were considered as biodegradable or bioinert, but there still with the risk of corrosion and emission of harmful element to human body. Hence, coating of calcium phosphate on the implants is believed to improve the stability of the implants and on the same time improve

the bone regeneration since the calcium phosphate compound able to form new bone tissue on the implants. In order to improve the performance of implants, there were some research has been investigated so far to consider calcium phosphate compound as coating materials. Table 2.6 below has shown the example for different coating materials on different implants.

Table 2.6: List of calcium phosphate coating on different implant material

No.	**Implants Materials**	**Coating Materials**	**Finding**	**Reference**
1.	Magnesium (Mg)	Dicalcium Phosphate Dihydrate (DCPD)	• DCPD coated scaffold has good mechanical properties, biocompatibility and suitable degradation rate • Stimulate bone regeneration	Zhang *et al*., 2022
2.	Magnesium (Mg)	Dicalcium Phosphate Dihydrate (DCPD) and Hydroxyapatite (HAp) composite	• DCPD stimulate the precipitation of HA and remain the degradability of system • HAp reinforce the corrosion resistance	Han *et al*., 2021
3.	Magnesium (Mg)	Zinc doped Dicalcium Phosphate Dihydrate (Zn-DCPD)	• Improve corrosion resistance • Zn able to enhance the anti-adherent bacterial rate	Zhao *et al*., 2016