SCHOOL OF MATERIALS AND MINERAL RESOURCES ENGINEERING

UNIVERSITI SAINS MALAYSIA

PRELIMINARY STUDY ON BIOCERAMICS CONTAINING GALLIC ACID

By

FARRAH IZZATUL AIN BT KAMARUDIN

Supervisor: Dr. Khairul Anuar Bin Shariff

Co-Supervisor: Dr. Mohamad Hafizi Bin Abu Bakar

Dissertation submitted in partial fulfilment

of the requirements for the degree of Master of Science

(Materials Engineering)

UNIVERSITI SAINS MALAYSIA

SEPTEMBER 2020

DECLARATION

I hereby declare that I have conducted, completed the research work nd written the dissertation entitled **"Preliminary Study on Bioceramics Containing Gallic Acid".** I also declare that it has not been previously submitted for the award of any degree or diploma of similar title as this for any other examining body or university.

Name of student: Farrah Izzatul Ain Bt Kamarudin Signature:

Date : 15 September, 2020

Witness by

Supervisor : Dr. Khairul Anuar Bin Shariff Signature:

Date : 15 September, 2020

ACKNOWLEDGEMENT

In the name of Allah, Most Generous and Most Merciful

It is with the deepest sense of gratitude and feeing blessed by the Almighty ALLAH who has bestowed in the strength and ability, that I am able to successfully complete this project. All good aspirations, motivation and prayers that have come out from this endeavour are due to His blessings.

Firstly, I wish to express my sincere gratitude and greatest appreciation to my supervisor, Dr. Khairul Anuar Bin Shariff and my Co-Supervisor Dr. Mohamad Hafizi Bin Abu Bakar from School of Industrial Technology, who has been patiently in providing supervision, guidance and support throughout the process of completing this project. His relentless effort and willingness to assist and teaching valuable knowledge that I will never forget.

My appreciation also goes to all the lecturers in the School of Materials and Mineral Resources Engineering, especially to my internal examiner, Dr. Shah Rizal Bin Kasim for her evaluation during my viva and her continued guidance after the process.

This thesis is also dedicated to the staffs of the School of Materials and Mineral Resources Engineering, for giving me the chance to use the equipment and apparatus at the laboratory. Many thanks to my friends and family members especially my beloved parents Kamarudin Bin Mohd Nazar and Fauziah Bt Che' Ros for their love, attention and supports. Lastly, my thanks to everyone who has contributed directly and indirectly towards the successful completion of this project.

TABLE OF CONTENTS

DECLARATION .... **i**

ACKNOWLEDGEMENT .... **ii**

TABLE OF CONTENTS .... **iii**

LIST OF TABLES .... **vii**

LIST OF FIGURES .... **viii**

LIST OF SYMBOLS .... **xi**

LIST OF ABBREVATIONS .... **xii**

ABSTRAK .... **xiii**

ABSTRACT .... **xiv**

CHAPTER 1 .... **1**

INTRODUCTION **1**

1.1 Background of Research **1**

1.2 Problem Statement **3**

1.3 Research Objectives **6**

1.4 Experimental design **6**

1.5 Thesis outline **7**

CHAPTER 2 .... **8**

LITERATURE REVIEW **8**

2.1 Introduction **8**

2.2 Introduction to different type of bone substitute. **13**

2.2.1 Autograft **14**

2.2.2 Allograft **16**

2.2.3 Xenograft 17

2.2.4 Alloplastic bone graft 19

2.3 Synthetic Bone Graft Substitute 20

2.3.2 Hydroxyapatite (HAp) 22

2.3.3 Alpha-tricalcium phosphate (α-TCP) 23

2.3.4 Beta-Tricalcium phosphate (β-TCP) 24

2.4 Introduction of Gallic acid as Bioactive Compound in bioceramic materials 26

2.4.1 Gallic Acid synthetic derivatives. 27

2.5 Application of Bioactive compound and its derivative 28

2.5.1 Anti-Cancer 30

2.5.2 Effect of Gallic Acid in accelerating and inducing new bone regeneration. 31

2.5.3 Recent studies to improve bone regeneration of bioceramic materials using natural compound. 32

2.5.4 Advantage of natural compound as coating materials. 32

2.5.5 Method to coat natural compound on the surface of bioceramic materials. 32

2.6 Research idea 33

CHAPTER 3 34

MATERIALS AND METHOD 34

3.1 Introduction 34

3.2 Raw materials and Chemicals. 34

3.2.1 Calcium Carbonate powder($CaCO_3$) 34

3.2.2 Dicalcium phosphate dihydrate powder (DCPD) 34

3.2.3 Ethanol solution 35

3.2.4 Gallic acid 35

3.2.5 Deionized Water (DIW) 35

3.3 Fabrication of different types bioceramic pallet. 36

3.3.1 Fabrication of Bioceramic pellet 36

3.4 Preparation of gallic acid solution. 38

3.5 Fabrication of different type bioceramics with GA solution. 40

3.6 Characterizations 42

3.6.1 Physical appearance observation 42

3.6.2 Phase analysis 43

3.6.3 Morphological observation 43

3.6.4 Surface topography analysis. 43

3.6.4 Functional Group Test 44

3.6.5 Hardness test (Vickers) 45

CHAPTER 4 46

RESULTS AND DISCUSSION 46

4.1 Introduction 46

4.2 Characterizations of raw materials 47

4.2.1 Physical appearance of raw materials 47

4.2.2 Morphology observation 48

4.2.3 Phase analysis 49

4.2.4 Functional group analysis 50

4.3 Characterization of Bioceramic pallets 52

4.3.1 Physical appearance 52

4.3.2 Phase analysis 55

4.3.3 Morphology observation 56

4.3.4 Functional group analysis 58

4.3.5 Vickers microhardness test 59

4.4 Characterization of different type of bioceramic containing gallic acid 61

4.4.1 Effects of soaking time. 61

4.4.2 Effects of Gallic Acid concentration 76

CHAPTER 5 93

CONCLUSION AND RECOMMENDATION 93

5.1 Conclusion 93

5.2 Recommendation 94

REFERENCES 95

APPENDICES 105

LIST OF TABLES

Table 2. 3: Fracture healing and bone morphogenesis terminology (Nyan, 2016).... 10

Table 2. 4: Calcium phosphate salts group compounds (Rojbani *et al.*, 2011).......... 11

Table 2. 6: Types of bone graft materials (Cho et al., 2017) 13

Table 3. 1: The amount of GA powder used to make different GA concentration solution.. 39

Table 4. 1: Physical size of bioceramics. 53

Table 4. 2: The hardness of different type of bioceramics.. 60

Table 4. 3: Bioceramic weight before and after soak in GA solution with different amount of soaking time. 62

Table 4. 4: Density of bioceramics before and after soaked in GA solution with different amount of soaking time 62

Table 4. 5: The hardness of bioceramics after soaked in GA solution with different soaking time 75

Table 4. 6: Bioceramic weight before and after soaked in GA solution with different concentration 78

Table 4. 7: Density of bioceramics before and after soaked in GA solution with different concentration 78

Table 4. 8: The bioceramics hardness after soaked in GA solution with different concentration 92

LIST OF FIGURES

Figure 2. 1: Hierarchical organization of bone tissue (Ferreira et al., 2018). 8

Figure 2. 2: Bone-derived factors (BDFs) system (Su et al., 2019) 9

Figure 2. 3: Calcium phosphate-based applications (Jeong et al., 2019) 12

Figure 2. 4: Recent progress in regenerative periodontal and bone therapies (Hasan *et al.*, 2018).. 15

Figure 2. 5Fresh Frozen Allograft Bone plus Bone Marrow Aspiration (Ferreira et al,. 2018).. 16

Figure 2. 6: Nonunion of the xenogenous bone graft clinically (Shibuya and Jupiter, 2015).. 18

Figure 2. 7: Application of CaP as bone graft (Bansal *et al.*, 2009)........................ 21

Figure 2. 8: The used of HAp bioceramic for dentistry (Bharwani et al., 2012)..... 22

Figure 2. 9: Scanning electron micrograph of porous **α-TCP** particles: (a) low-magnification image; (b) high-magnification image (Yoshiya Hashimoto et al,. 2018).. 23

Figure 2. 10: Improved Delivery Vehicle (Allison et al., 2011).............................. 25

Figure 2. 11: The fruits contains natural phenolic of Gallic Acid (Dludla et al., 2019) 26

Figure 2. 12: Chemical structure of GA (Locatelli et al,. 2013).............................. 27

Figure 2. 13: Gallic acid reaction toward cell. A: Negative control group, B: Positive control group, C: Gallic acid powder group, D: Gallic acid liposome group. 28

Figure 2. 14: Important uses of gallic acid and its ester derivatives (Choubey et al., 2015).. 29

Figure 2. 15: The proposed signalling pathways for GA-inhibited migration and invasion of pc-3 human prostate cancer cells in vitro (Liu et al., 2011). 30

Figure 2. 16: Role of the polyphenols in dynamics of bone tissue homeostasis (Dudarić et al., 2015)... 31

Figure 3. 1: Steps involved in fabrication of different type of bioceramic.............. 37

Figure 3. 2: Preparation of bioactive Gallic Acid solution...................................... 39

Figure 3. 3:Experimental set up for biocompatibility study between bioceramic and bioactive compound. 40

Figure 3. 4: a) Low concentration of GA solution b) Saturated GA solution 41

Figure 3. 5: The Vickers microhardness working principle. 45

Figure 4. 1: Physical appearance of a) $CaCO_3$ b) DCPD c) Gallic Acid 47

Figure 4. 2: The SEM image of a) CaCO3 b) DCPD c) Gallic Acid under magnification 2.5K x... 48

Figure 4. 3: XRD pattern of DCPD, $CaCO_3$ and GA powder 49

Figure 4. 4: FTIR analysis of raw materials 51

Figure 4. 5: Physical appearance of different type bioceramic pellet..................... 54

Figure 4. 6: XRD pattern of bioceramic specimens. 55

Figure 4. 7: SEM micrograph of different type of bioceramic pallets surface 57

Figure 4. 8: FTIR analysis for bioceramic pallets after sintering........................... 58

Figure 4. 9: Microhardness value for different type of bioceramics 60

Figure 4. 10: Physical appearance of bioceramics with different soaking time in Gallic acid concentration.. 63

Figure 4. 11(a): SEM image of Hydroxyapatite (HAp) after soaking in GA solution. 66

Figure 4. 12: XRD pattern of bioceramic in GA solution under different soaking time. 70

Figure 4. 13: FTIR analysis for HAp in GA solution with different soaking time.. 72

Figure 4. 14: Bioceramics hardness results for different soaking time in GA solution 75

Figure 4. 15: Physical appearance of bioceramics pellets after soaking different GA concentration. 79

Figure 4. 16 (a): SEM image of Hydroxyapatite (HAp) after soaking in different GA solution concentration. 81

Figure 4. 17: The XRD pattern of bioceramics in different GA solution concentration. 85

Figure 4. 18 (a): FTIR analysis of HAp bioceramic in different GA solution concentration. 87

Figure 4. 19: The AFM topography image of bioceramics. 90

Figure 4. 20: Bioceramics hardness results after soaked in different GA concentration. 92

LIST OF SYMBOLS

Min	Minutes
C	Celcius
g/mol	Gram/mol
β	Beta
A	Alpha

LIST OF ABBREVATIONS

BDFs	Bone derived factors
CaP	Calcium Phosphate
DCPD	Dicalcium phosphate anhydrate or monetite
GA	Galic acid
HAp	Hydroxyapatite
HCV	Hepatitis C virus
α-TCP	α-Tricalcium phosphate
β-TCP	β-Tricalcium phosphate

KAJIAN AWAL TERHADAP BIOSERAMIK MENGANDUNGI ASID GALLIK

ABSTRAK

Tujuan kajian ini adalah untuk mengkaji kebarangkalian Asid Gallik untuk digabungkan ke dalam tiga jenis bahan bioseramik yang berbeza seperti HAp, α-TCP dan β -TCP. Bagi mencapai tujuan ini, tempoh rendaman bioseramik di dalam larutan asid gallik dan kepekatan asid gallik akan dipelbagaikan bagi memahami faktor-faktor ini semasa penyediaan bioseramik mengandungi asid gallik. Komposisi, morfologi permukaan, kumpulan berfungsi, topografi permukaan dan kekerasan spesimen akan dianalisa menggunakan Pembelauan X-Ray (XRD), Mikroskop Imbasan Elektron (SEM), Sinaran Inframerah Transformasi Fourier (FTIR), Mikroskopi Berdaya Atom (AFM) dan Ujian Kekerasan Vickers. Hasil kajian menunjukkan bahawa kehadiran asid gallic dalam spesimen bioceramic tidak dapat dikesan walaupun telah direndam dalam larutan asid gallik selama 5, 10 dan 15 minit tetapi penambahan berat dan ketumpatan telah dikesan berlaku kepada HAp bioseramik. Sebaliknya, apabila kepekatan larutan asid gallik dinaikkan sehingga 0.15 mol / L, pembentukan asid gallik Kristal dapat dikesan pada permukaan a-TCP dan b-TCP seteleh direndam di dalam larutan ini selama 5 minit. Tambahan lagi, bioseramik HAp menunjukkan peningkatan dari segi berat dan ketumpatan, sementara keputusan yang berbeza telah diperolehi bagi α -TCP dan β -TCP. Selain itu, faktor keliangan yang berbeza bagi bioseramik merupakan peranan yang penting yang berkemungkinan hadir disebabkan oleh suhu pembakaran yang berbeza bagi setiap bioseramik. Oleh itu, kajian awal ini menunjukkan beberapa pandangan mengenai parameter yang perlu dikaji untuk menggabungkan bahan bioseramik dan asid gallik.

PRELIMINARY STUDY ON BIOCERAMICS CONTAINING GALLIC ACID

ABSTRACT

The aims of this study are to investigate the probability of Gallic Acid to be introduced into different type bioceramic materials such as HAp, α -TCP and β -TCP. To achieve this aims, soaking time of bioceramics in gallic acid solution and concentration of gallic acids were varies to understand these factors during preparation of bioceramics containing gallic acids. The compositional, surface morphologies, functional group, surface topography and hardness of specimens were analysed using X-Ray Diffraction (XRD), Scanning Electron Microscope (SEM), Fourier Transform Infrared (FTIR), Atomic Force Microscope (AFM) and Vickers hardness test, respectively. The results obtain showed that the presence of gallic acid in bioceramic specimens cannot be detected even soaking in gallic acid solution for 5, 10 and 15 mins but there is increasing of HAp weight and density after soaking in Gallic acid solution. On contrary, when increasing the concentration of gallic acid solution up to 0.15 mol/L, the formation of Gallic crystals was observed using SEM on the surface of α-TCP and β -TCP after exposing with this solution for 5 mins. Moreover, HAp bioceramic shows gaining in term of weight and density while α-TCP and β -TCP shows opposite result. Besides, the different in bioceramics porosity also play crucial role which may occur due to different sintering temperature of each bioceramic. Therefore, this preliminary study shows some insight regarding the parameters that need to be adjusted in fabricating bioceramic materials containing gallic acid.

CHAPTER 1

INTRODUCTION

1.1 Background of Research

For the past 20 years, bioceramic materials had been widely used as bone substitute materials in clinics and hospitals due to its good biocompatibility (Xu *et al.*, 2017) and osteoconductivity (Rojbani *et al.*, 2011). However, recent research trend of bioceramic materials more focused to improve the quality of life for patients who suffered from chronic diseases especially that related to bone defect (Grandi *et al.*, 2011) such as osteomyelitis (Vugt *et al*,. 2016) and cancer (Macedo, 2017). Therefore, comprehensive studies have been done to date in order to enhance bioceramic materials application as bone replacement materials.

The bioceramic that was made from calcium phosphate product usually been designed able to mimic the surface porosity and grain structure of replacement materials (Parsons *et al.*, 2010). Bioceramics used as bone materials replacement, had been design with ability to interact with body's biological systems (Ginebra *et al.*, 2018) which is also known as osteointegration as reported by Wang and Yeung, (2017). The bone grafting materials was divided into natural transplants (autografts, allografts and xenografts) and synthetic materials (alloplasts) (Sheikh *et al.,* 2015).

Autografts type bone grafting was harvested and being transferred to the same individual. Next, was allograft bone grafting which genetically had been taken non identical members with ability to produce in massive quantity and avoiding a second surgical site. Bone grafting using the Xenografts were acquired from species other than human than compatible to human (Sheikh *et al,.* 2015). Alloplastic bone graft replacement can be produce in variant mineralized layer, surface texture with many size and shape and naturally allowed cells formation (Titsinides *et al,.* 2019).

Calcium phosphate bioceramic materials is one of the popular bone substitutes that frequently use in both orthopaedics and dentistry application (Eliaz and Metoki, 2017). The bioceramic materials that was made up from calcium phosphate cements such as Alpha-tricalcium phosphate (α-TCP), Beta-Tricalcium phosphate (β-TCP) and hydroxyapatite (HAp) able to offer numerous advantages. This type of material was popular to be use as bone substitute due to its biocompatibility (Borodajenko *et al,.* 2012) and bioactivity properties (Thürmer, Diehl and dos Santos, 2016) . In addition, as reported by previous study done by Chung, *et al,.* (2019) it also frees from immunogenic response, safe from disease transmission and nontoxic. On top of that, this type of bioceramic material had been widely use in biomedical application due to its great osteoconductive properties and show excellent properties in bone tissue engineering applications and even as drug delivery systems (Turon et al., 2017).Other than that, (α-TCP), (β-TCP) and (HAp) has the structure that able to resemble the native bone and have similar composition to bone mineral (Pokhrel, 2018).

In this recent year, gallic acid (GA) had been play an important role in the medicalinal practice due to its beneficial effect to human's health (Kahkeshani *et al.*, 2018). GA is a bioactive compound that was derived from a group of polyphenol compounds. This GA compound was widely used due to its anti-inflammatory effect, anti-oxidative, anti-cancer and been reported to have an anti-osteoporosis effect as reported by Jin *et al.*, (2015). In addition, based on the research done by Kahkeshani *et al.,* (2018) this type of phytochemicals also contains cardioprotective, gastroprotective, and neuroprotective effects.

Based on the latest trend and study, the application of bioactive compound hadhad shown positive trends. The usage of bioactive compound like GA had been widely used especially in medical industry, pharmacology, packaging, food conservation and cosmetics (Cazzola *et al.,* 2019). Currently, GA had been widely to treated various type of cancer such as breast cancer (Khorsandi *et al.,* 2020), prostate cancer (Liu *et al.,* 2011), bladder cancer (Liao *et al.,* 2018), cervical cancer (Aborehab and Osama, 2019). Besides, GA can also be grafting onto metal implant (Bioactive Ti6Al4V) to enhance its biological response in bone contact applications (Cazzola *et al.,* 2019).

1.2 Problem Statement

Calcium phosphate (CaP) bioceramics are excellent synthetic bone substitute materials which able to be fabricated to hydroxyapatite (HAp), alpha-tricalcium phosphate (α-TCP) and beta-tricalcium (β-TCP) (He *et al.,* 2017). Due to their great biocompatibility and osteoconductivity it had been widely used in clinics and hospital (Ngoc, 2012). However, if additional properties of bioceramic materials can be added on top of biocompatibility and osteoconductivity properties, it applications in clinics and hospitals can be broader and varied the applications of this materials (Kim, 2016).

Our bone has an ability to self-repair and regenerate when bone was defect. The properties would trigger when bone had been in severe trauma, tumour resection, cancer. Other than that, congenital diseases may also cause the bone condition to be worsen which only able to be treat by bone grafting surgery that was replace using bone made up of certain type biomaterials (Gao *et al.*, 2017)**.** The bone grafting method use autografts, allografts, and or bone graft substitutes surgery method require an operation which function to replace and restore the loss of bone at the affected area (Brydone *et al.,* 2010)**.** In contrary, as reported by Bai *et al.,* (2018) this type of method can cause patient's body immune to reject foreign object which would commonly causing infection and delayed the wound healing (Friesenbichler *et al.*, 2014)

There are many studies had been reported to date about additional improvement of bioceramic materials for clinical applications. For example, study done by Nagaraj and Samiappan, (2019) had reported ability to introduce antibacterial properties of HAp bioceramics by biomimetization this ceramic with Azadirachta Indica (AI) to promote antioxidant, antibacterial, and anti-inflammatory potential for lipopolysaccharide (LPS). Next, (α-TCP) bioceramic is sintered together with iron (Fe) with purpose to improve its strength and toughness by adding biocompatible metallic component that was induce using spark plasma sintering (Casas-Luna *et al.*, 2018). Meanwhile, (β-TCP) bioceramic were improved by introduce active ions strontium and zinc that used as structural modification to increase its flexibility properties (Boanini *et al.*, 2019).

The bioactive compound like GA can be used to be incorporated onto the bone graft bioceramic material due to their favourable properties on bone growth. This material contain bioactive phenolic compound which was acknowledge benefit to health due to their antioxidant and anti-inflammatory properties (Dudarić *et al.*, 2015). On top of that, as report by (Choubey *et al.*, 2015) GA contain strong and natural antioxidative, anti-allergic, anti-arteriosclerosis and play important role to prevent formation of cancer development. Furthermore, the polyphenols compound in the GA able to promote of healthy osteoblasts and enhance the apoptosis of osteosarcoma cells.

The research done by Kharouf *et al.*, (2020) bioactive compound like polyphenols was used in dental field by induce it in the formation of hydroxyapatite to modify the surface of enamel and provide better adhesion and improving its antimicrobial properties. Bioceramics that was used as scaffold shows improvement in bioactivity when incorporate with plant derived substances. The phytochemicals in plants (Chen *et al.*, 2018) acts as cell growth factors by promoting production of protein that responsible for osteoconductive activities. The study done by Cazzola *et al.*, (2019) reported that the ability to fabricate surface of hydroxyapatite through in vitro deposition process using natural polyphenol biomolecules and had been established able to speed up regeneration of bone growth and density.

Therefore, in this study three types of different bioceramic pallet which were HAp, α-TCP and β-TCP been used to study the incorporate effect with Gallic Acid (GA) stock solution which act as bioactive compound. The study was performed by Mas and Borrós, (2016) exposing surface of HAp, α-TCP and β-TCP bioceramic pallet by soaking it in 5 minutes different time setting soaking time show an optimise results so, the study was continuing and done by using 5, 10 and mins soaking time.

In addition, the research was then proceeded by varying the GA stock concentration to observed the limit GA incorporated amount as the concentration increase. The concentration used was 0.05, 0.10 and 0.15 mol/L based on the study done by. The soaking technique process was used due to the ability to form bioactive coating at low temperature (Boix *et al.*, 2015) in order to prevent thermal denaturation of GA with melting point, Tm 62.62 ^{o}C (Nematollah *et al.*, 2014).

1.3 Research Objectives

1. To evaluate the effect of soaking time in fabricating different type of bioceramics that containing bioactive compound (Gallic Acid).

2. To investigate the effect of Gallic acid concentration in fabricating bioceramics materials containing Gallic Acid.

1.4 Experimental design

To study the biocompatibility study of bioceramic toward gallic acid, three different type of bioceramics were fabricated. The HAp, α-TCP and β-TCP were form in same pallet size shape. This bioactive GA powder was diluted using deionised water (DIW). Each bioactive solution was prepared by diluting 50 mg of GA powder with 3 ml of DIW in 5 ml volume of falcon tube. Next, the solution was mix and stirring by using Vortex shaker for 30 mins to produce fully dissolved clear solution. Setting Ssoaking time was fixed to 5, 10, and 15 mins for each type of bioceramic. Then, the labelled pallet was dry in room temperature. For further investigation on different variable, another set of bioceramic pallet sample was soaked different in GA solution concentration (0.05, 0.10 and 0.15) mol/L to analyse the materials biocompatibility.

For characterization of each sample analysis, X-ray Diffraction (XRD) was used to evaluate the changes of bioceramic phases before and after soaking in GA solution. The morphology of soaked bioceramic sample was analysed using Scanning Electron Microscopy. Moreover, SEM was also used to check the surface morphology and the bridging crystal between granules. The Fourier Transform Infrared Spectroscopy (FTIR) was used to perform compositional on the sample by detecting some specific functional groups.

1.5 Thesis outline

This thesis outline was organized into five chapters. The first chapter cover the research background, problem statement, objective and scope of research. In second chapter literature review on different type of bone grafting and bioceramic materials, previous study on induce other materials onto the bioceramic, literature review on gallic acid and its medicinal advantages. Chapter 3 explains the materials used, the experimental procedure parameter conducted and its characterization. The results and discussion are discussed in chapter 4. Finally, chapter 5 concludes the research and recommendation needed.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Bone disease are condition, which included any kind of diseases or injuries that affect human bones and may triggered abnormalities in human skeletal system. Bone fracture phenomenon was determined to one of the common causes of bone disease and some disease could also cause the bone to fracture (Chivima, 2013). Bone defects such as congenital deformities, tumor and cancer can be reconstructed by orthopaedic surgeons using certain protocols and processes (Khan *et al.*, (2012) (Wu *et al.*, 2018) (Chong, 2015)).

Formation of skeletal system was constructed from bone that made up from connective tissue, bone tissue an in Figure 2.1 and several type of mineral. The rigid and hardness of bone was generated from deposition of minerals like CaP and carbonate that function to protect vital organs maintain body shape and allows movement of the body (Ferreira *et al.*, 2018).

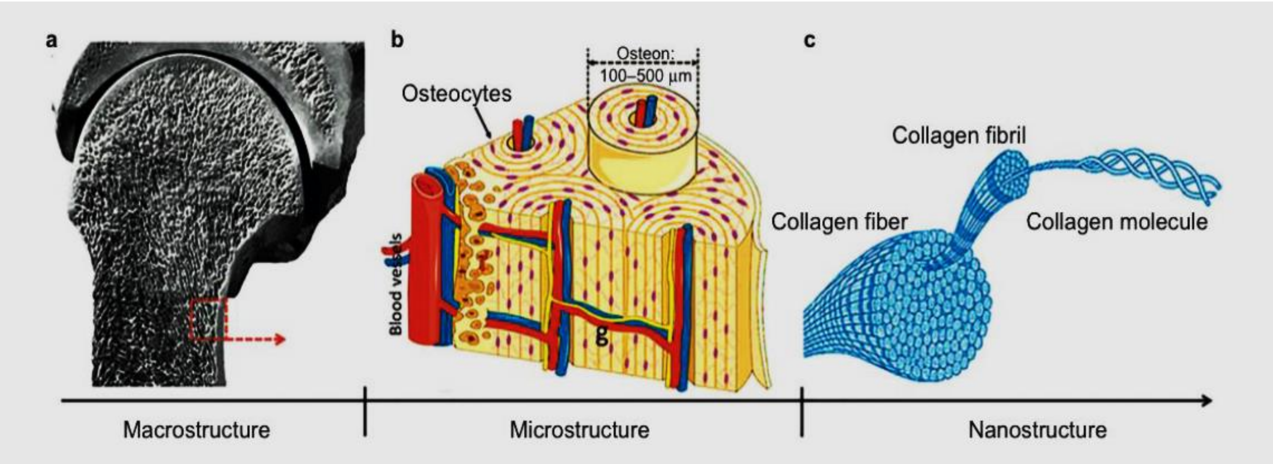

Figure 2. 1: Hierarchical organization of bone tissue (Ferreira et al., 2018).

The Bone derived factors (BDFs) as shown in Figure 2.2 help to regulate the bone function (Su *et al.*, 2019). As reported by Silva *et al.*, (2015) bone tissue was continuously been reproduced with the collaborated actions made by bone cells. Bone remodelling process involved bone resorption by osteoclast, bone formation by osteoblast and orchestrators to remodel the bone as shown in Figure 2.2 and the fracture healing terminologies as shown in Table 2.1

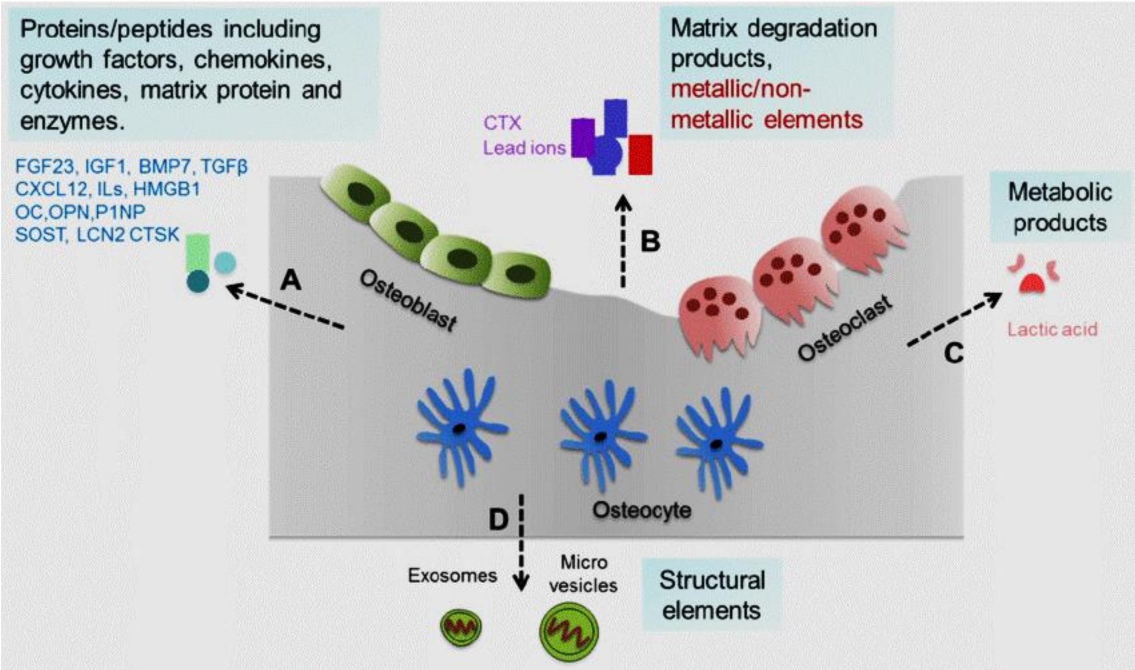

Figure 2. 2: Bone-derived factors (BDFs) system (Su et al., 2019)

Table 2. 1: Fracture healing and bone morphogenesis terminology (Nyan, 2016)

Term	**Definition**	**Examples**
Osteogenic	Contains Osteoprogenitor cells or stimulates (committed) osteoprogenitor cell proliferation	Bone marrow, bone graft
Osteoconductive	3-D scaffold that committed osteoprogenitor cells produce bone; supports ingrowth of capillaries and cells from the host bed; guides repair in the location where normal healing will occur if left untreated	Demineralized bone matrix, bone graft, calcium phosphate ceramics
Osteoinductive	Bone morphogenesis: phenotypic conversion of stem cells from a non-osseous environment to chondrocytes and osteoblasts; allows repair in a location that would not normally heal if left untreated; able to induce bone formation at an ectopic (extra-skeletal, non-osseous) site	Bone graft, dentine matrix, demineralized bone matrix, bone morphogenetic proteins (BMPs)

The study made by Barrère *et al.*, (2006) showing calcium phosphate had massively been used for dental and orthopaedics application. This type of bioceramic able to produce porous type of scaffold which made this material gain popularity in bone tissue engineering field. The outstanding properties was it able to stimulate bone formation and bone bonding and provide excellent interaction with extracellular fluids and cellular activity. Table 1 show the calcium phosphate salts group of compounds. Even though, product was made from the same materials, the properties of the product can be change when using different composition and crystal structure (Rojbani *et al.*, 2011) as shown in Table 2.2 .

Table 2. 2: Calcium phosphate salts group compounds (Rojbani *et al.*, 2011)

Name	**Formula**	**Abbreviation**	**Ca/P**
Dicalcium phosphate anhydrate or monetite	$CaHPO_4$	DCPA	1.00
Dicalcium phosphate dihydrate or brushite	DCPD	DCPD	1.00
Octacalcium phosphate	$Ca_8(PO_4)4(HPO_4)2.5H_2O$	OCP	1.33
Tricalcium phosphate	Ca3(PO4)2	TCP	1.50
Hydroxyapatite	Ca10(PO4)6(OH)2	HA	1.67

Bioceramics that was used as bone grafting scaffold need to be osteoconductive which primarily function to promote osteosynthesis, that mean it had ability to fix problem at aeffected bone. Since the bone graft was fabricated from synthetic materials, continuous research had been done to improve the quality and the properties of the bioceramic. In fact, recent study proves the ability of bone graft substitute in different shape as shown in Figure 2.3. , from pallets and solid blocks, to injectable and mouldable putty (Jacobs, 2017).

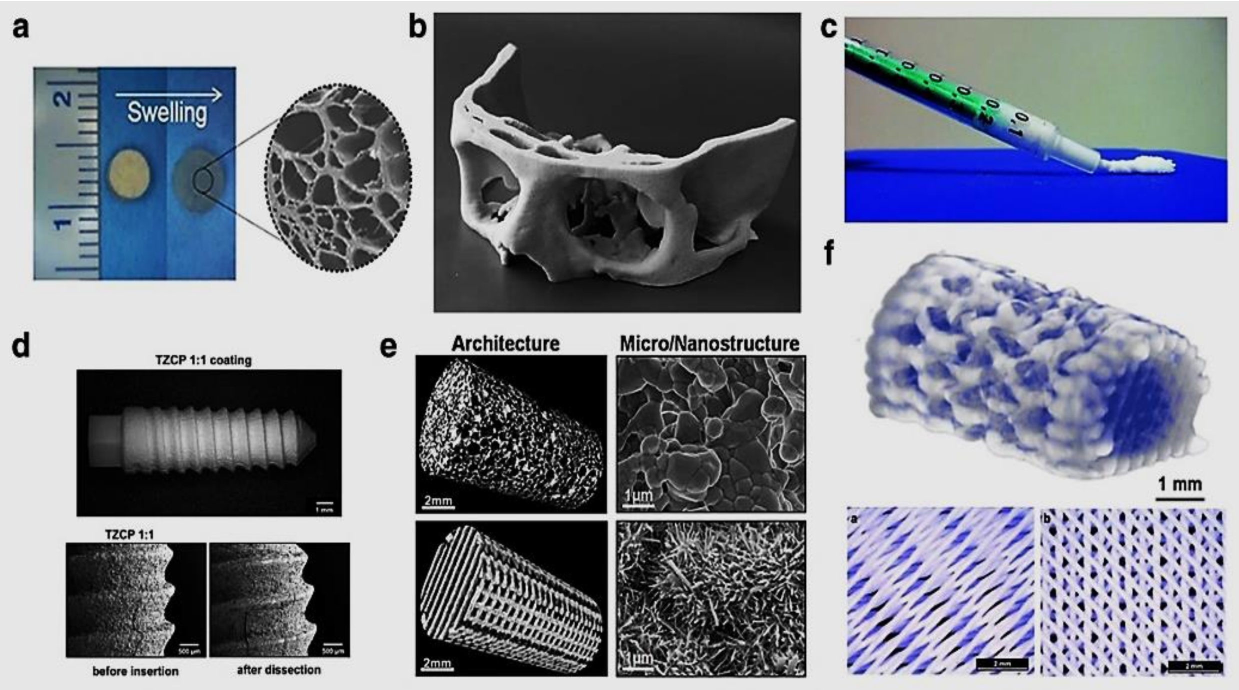

Figure 2. 3: Calcium phosphate-based applications (Jeong et al., 2019)

2.2 Introduction to different type of bone substitute.

Alternative materials had been widely produced purposely function to replace the missing bone in the human body especially. To promote introduction of new bone formation inside the bone defect area, the bone substitute materials. The bone graft materials were differently divided into autograft, allograft, xenograft, and alloplasts as show in Table 2.3, which act as a mineral reservoir to assist new bone formation (Cho et al., 2017).

Table 2. 3: Types of bone graft materials (Cho et al., 2017)

Type	**Materials**
Autograft	Extraoral: (Iliac Crest, Tibia, Fibula, Ribs) Intraoral: (Chin, Exostosis, Ramus, Tuberosity)
Allograft	Mineralized (FDBA) and demineralized freeze-dried bone allografts (DFDBA)
Xenograft	Bovine derived, porcine derived, equine derived
Alloplasts	Hydroxyapatite, calcium phosphate, β-TCP, bioactive glass, synthetic glass

2.2.1 Autograft

The autograft bone replacement type was the type of bone grafting that was receive from the same individual that receiving the graft. It is also known as autologous or autogenous where it has less risk of rejection since it was obtained originated from patient's body. In contradict aspect, an additional of surgical site was required which may adding another location for post-operative pain and complication (Ngoc, 2012).

Autograft bone graft had been considered as one of the excellent bone graft type since the process was done by incorporates osteogenic cells and an osteoconductive mineral matrix. The autograft bone replacement may needed an operation to be done by removing some of patient's bone to replace defect bone at another part of body (Brydone *et al,.* 2010) as shown in Figure 2.4. However, the autograft disfavour properties would cause further bone damage, prolonged period of anaesthetic, risk of morbidity and also would cause it to failure (Zhang *et al.*, 2020).

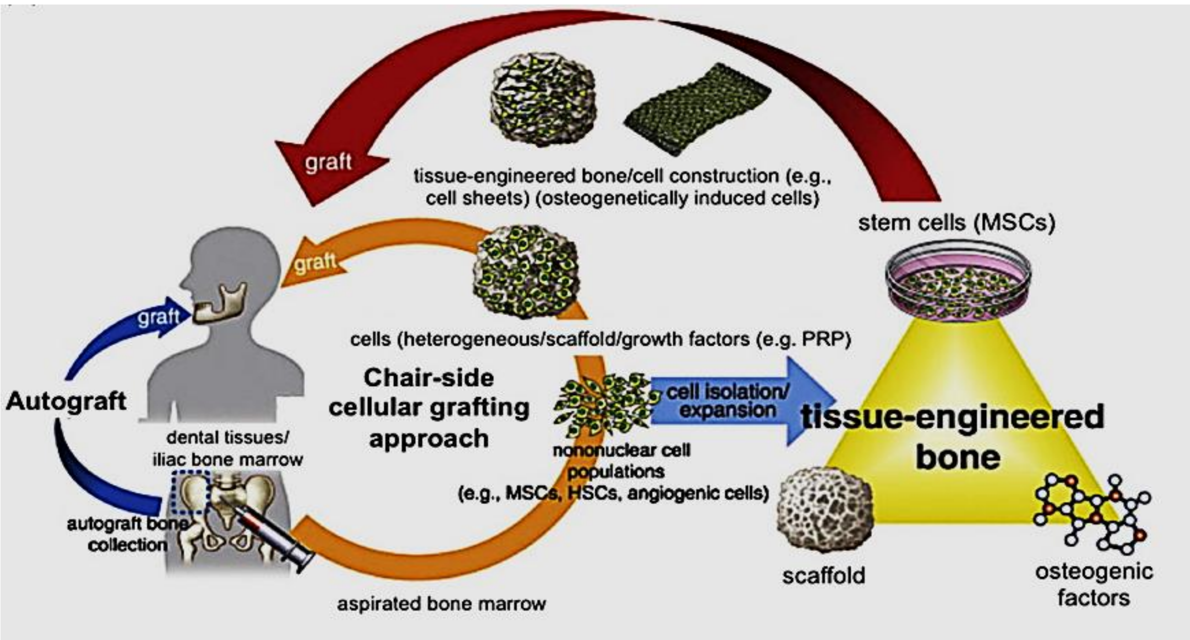

Figure 2. 4: Recent progress in regenerative periodontal and bone therapies (Hasan *et al.*, 2018)

2.2.2 Allograft

The allograft bone graft method was almost the same as autograft but, the bone source was taken from another human being and being harvest in another person body. The bone was typically being donated and it was kept in the bone bank which can be donated to the needed patient (Ngoc, 2012). Based on the information written by Sohn *et al*,. (2019) paper the limitation and infection risk that was faced by autogenous bone graft had led to the alternative way of using allograft bone graft that was performed by having the donor screening test to minimized the infection and complication risk. The allograft bone was commonly preserved using freeze drying process as shown in Ffigure 2.54 of fresh frozen bone marrow and vacuum-packing process.

Figure 2. 5Fresh Frozen Allograft Bone plus Bone Marrow Aspiration (Ferreira et al,. 2018)

The disadvantage of allograft was the limited amount of supply, could cause antigenic response and the worst could be potential of disease transmission like HIV and hepatitis C virus (HCV) that might be transmitted with allograft bone (Allison *et al.*, 2011). On top of that, the study reported by (Sohn *et al*,. 2019) this process might also affect the mechanical properties that able to weaken the bone where some living osteogenic cell had been removed in order to sterilize the allograft bone.

2.2.3 Xenograft

The Xenograft was obtained from another type of species other than the human being for example bovine. It is also only being distributed in a form of calcified matrix. Xenograft bone substitute source supplies usually obtain from animal species. Due to this usually this bone type grafting available in unlimited amount since it was taken and supplied from healthy animal donors with controlled biology to avoid any risk of human disease transmission which would able to fulfil the demand supply for bone graft product (Bracey *et al.*, 2018). As reported by (Shibuya and Jupiter, 2015) this type of bone graft had abundance amount of donors and can be less costly and more readily available with much longer shelf life. However, Shibuya and Jupiter, (2015) reported that this type of bone graft species had showed a poor incorporation rate. The Figure 2.6 shows the condition of patient's foot after 6 months of surgery.

Figure 2. 6: Nonunion of the xenogenous bone graft clinically (Shibuya and Jupiter, 2015)

2.2.4 Alloplastic bone graft

The Alloplastic graft bone substitute method commonly was made from hydroxyapatite where it is a natural occurring mineral that recognise as main mineral component in bone. Furthermore, due to the osteoconduction (Kumar *et al.*, 2016) hardness and ability to blend well with bone this type of mineral been widely used as synthetic bone graft replacement (Glogauer *et al*,. 2015).

Alloplastic Materials Alloplastic materials are synthetic, inorganic, biocompatible, and/or bioactive bone graft substitutes, which are claimed to promote bone healing through osteoconduction. The available alloplastic materials are Plaster of Paris, polymers, calcium carbonate, and ceramics. Ceramics can be classified into resorbable (e.g., tricalcium phosphate and resorbable HA) and nonresorbable (dense HA, porous HA, and bioglass). Bioglass developed by Hench is one of the latest and promising substitutes for bone graft materials. Various alloplastic materials are illustrated in the following sections.

The bone replacement that was made from Alloplastic materials can be form in various type of shape and size. The advantage of alloplastic graft materials it easy to be grafted onto patient affected bone since it can be fabricate according to the patient requirement (Grandi *et al.*, 2011). On top of that, this type of bone graft was also commonly used in dentistry field and made up from polymer, bioactive glass and also calcium phosphate. Calcium phosphates products usually being fabricate to form hydroxyapetite HAp and beta-tricalcium phosphate (TCP) since it able to resemble composition of bone (Kokosis, 2020).

2.3 Synthetic Bone Graft Substitute

The bone graft substitute had been used to treat bone defects especially in for human being. The bone grafting had been widely used to fill back the bone damage that may be cause by fracture, trauma, tumour resection or arthrodesis. In some cases, the bone graft may cause complication at the harvest site. The common reported complication that occurs can be infection, hematoma, nerve injury and fracture (Chung, Kim and Chung, 2019). Engineer improvement was important for bone graft before any bone graft substitute operation been performed to prevent any operation complication (Chhabra *et al*,. 2019)**.** Hydroxyapatite (HAp) and Tricalcium phosphate (TCP) are belong to the class of bioceramic materials. This type of materials had proven their ability in numerous orthopaedics field as shown in Figure 2.7 shows the usage of Calcium phosphate used as bone graft.

The HAp, Alpha (α-TCP) and beta-Tricalcium phosphate (β-TCP) had different purpose of usage even they have similar chemical structures. This phenomenon occurred due to the differ in their crystalline structure which resulted in different absorption characteristic. (Grandi *et al.*, 2011) reported the solubility rate of β-TCP is smaller than that of α-TCP but still higher than HAp. The HAp bioceramic was also commercially used in dental application due to its low solubility rate but has highest chemical stability (Kolmas *et al*,. 2019).

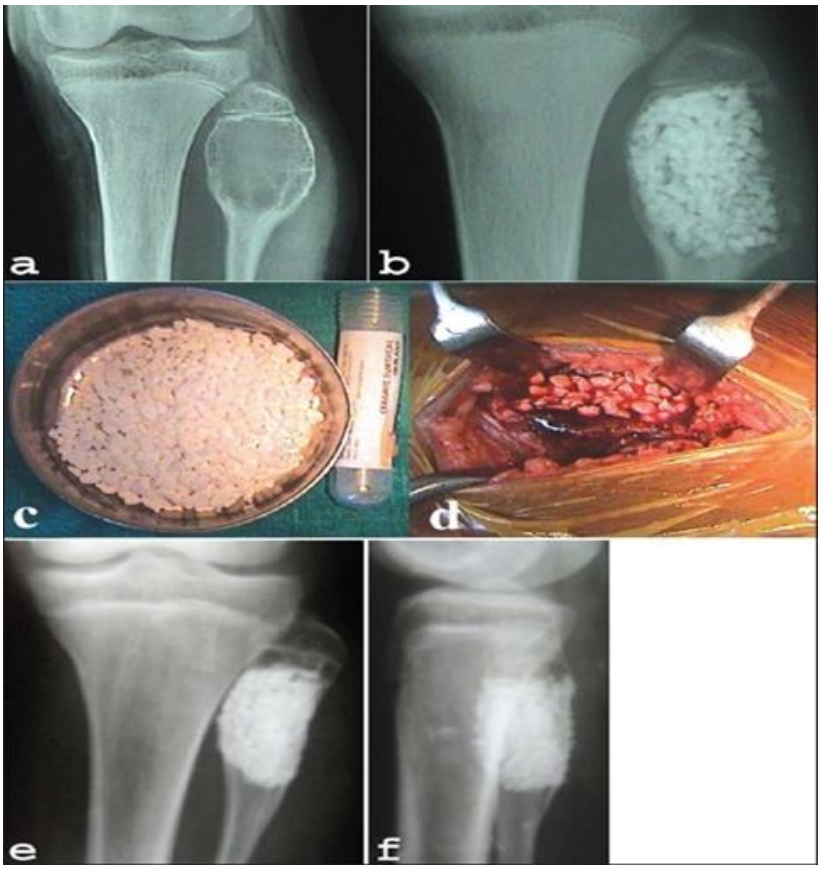

Figure 2. 7: Application of CaP as bone graft (Bansal *et al.*, 2009)

2.3.2 Hydroxyapatite (HAp)

Hydroxyapatite particle (HAp, $Ca_{10} (PO_4)_6 (OH)_2$) is the main inorganic constituent as a bone and teeth substitute. HAp bio ceramic have a great potential due to the excellent combination of properties such as enhancement of biocompatibility and reinforcing effect provided by the inorganic component (Turon *et al.*, 2017). HAp bioceramic surface structure able to imitate almost 60-70% human bone mineral phase (Othman and Zakaria, 2011).

On top of that, another form of HAp was in the nano form particle where it plays important role in variety biomedical such as dentistry application as shown in Figure 2.8. due to it high surface area volume ratio. The ultra-fine structure of the nano HAp almost resemble to the natural bone. There few methods such as chemical precipitaion, spray drying, sol-gel and etc that can be used to synthesis nano HAp where collagen matrix with average 50nm embedded in natural bone and teeth (Miraz, Rafie and Nordin, 2017).

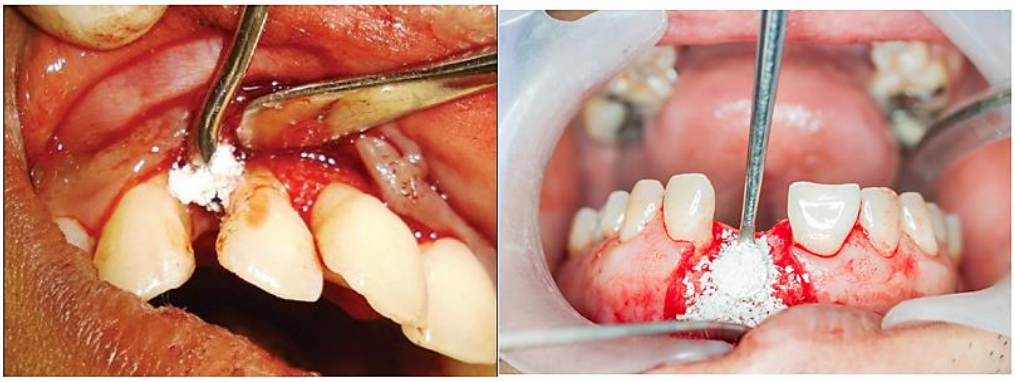

Figure 2. 8: The used of HAp bioceramic for dentistry (Bharwani et al., 2012)

2.3.3 Alpha-tricalcium phosphate (α-TCP)

α-TCP had been widely used for medical purpose for bone tissue regeneration due to its biocompatibility and biodegradability characteristics (Thürmer, Diehl and dos Santos, 2016). α-TCP is formed from β-TCP by heating it in the low temperature or also by thermal crystallisation of amorphous precursors with right amount of composition above the transformation temperature.

Furthermore, the α-TCP can rapidly absorb and replaced in the body where the degradation calcium phosphate material generated Ca^{2+}, PO_4^{3-}. The formation of Ca^{2+}, PO_4^{3-} substrates are required by the osteoblast differentiation and bone matrix mineralization (Liu *et al.*, 2015). Compared to β-TCP, the α-TCP is more soluble after heating the β-TCP to more than 1170°C (Medicine, 2016). Yoshiya Hashimoto *et al.*, 2018 reported that α-TCP able to biodegrade better due to the high solubility compared to HAp during the bone remodelling. Figure 2.9 show the SEM micrograph of α-TCP structure.

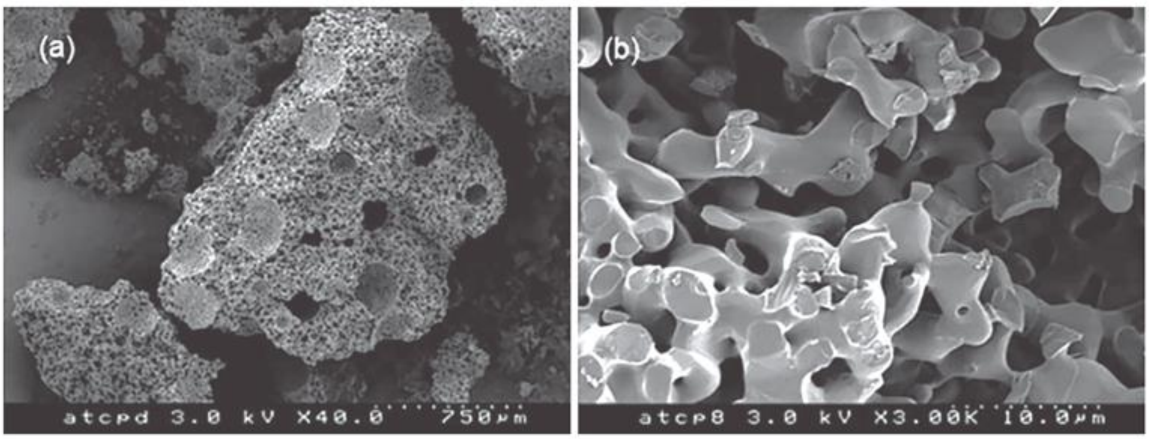

Figure 2. 9: Scanning electron micrograph of porous **α-TCP** particles: (a) low-magnification image; (b) high-magnification image (Yoshiya Hashimoto et al,. 2018)

2.3.4 Beta-Tricalcium phosphate (β-TCP)

β-Tricalcium phosphate (β-TCP) has the chemical formula $Ca_3(PO_4)_2$, with the Ca/P ratio was (1.:5). The chemical composition of β-TCP was close to the natural bone with good bioactivity and biodegradability with high dissolution rate in the human biological environment. This unique property of β -TCP play huge role in the biomedical materials since its easily be resorbed and replaced by natural bone (Sha *et al.*, 2011).

Based on the previous study report β-TCP was one of the calcium phosphate type that had been mainly used as the synthetic bone graft because it was nontoxic, deemed safe, safe from disease transmission, and free from immunogenic response. This type of bio-ceramic can be use at bone graft donor site with less risk of infirmity besides it is also fully biocompatible and bioactive (Chung, Kim and Chung, 2019).

However, as an osteoconductive materials, β-TCP had its own disadvantages and unfavourable properties. This bioceramic materials usually show poor absorption rate toward new bone. Next, the mechanical properties of this bioceramic was poor with slight brittleness that cause fatigue on material to occurred with inadequate holding control with lacks of osteoinductivity effect (Liu and Lun, 2012). The study done by (Allison *et al.*, 2011) state that β-TCP can be improve by modified it from solid block to injectable form as shown in Figure 2.10.