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SYNTHESIS OF POROUS CARBONATE APATITE/GELATINE SCAFFOLDS VIA FREEZE DRYING METHOD

By

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DECLARATION

I hereby declare that I have conducted, completed the research work and written the dissertation entitled "Synthesis of Porous CO_3Ap/Gelatine Scaffolds Via Freeze Drying Method". I also declare that it has not been previously submitted for the award of any degree or diploma or other similar title for any other examining body or University.

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LIST OF ABBREVIATIONS

CO_3Ap	Carbonate apatite
CO_2	Carbon dioxide
CPPD	Calcium pyrophosphate dihydrate
ECM	Extracellular matrix
FTIR	Fourier transformed infrared spectroscopy
HA	Hydroxyapatite
PVA	Poly vinyl alcohol
SEM	Scanning electron microscope
TCP	Tri-calcium phosphate
TTCP	Tetracalcium phosphate
XRD	X-ray diffraction

LIST OF SYMBOLS

Wt. %	Weight percent
%	Percent
˚C	Degree celcius
α	Alpha
β	Beta
μm	Micrometer
mm	Milimeter
g	Gram
˚	Degree
nm	Nanometer
Cm	Centimeter
kPa	Kilopascal
Mpa	Megapascal
ml	Mililiter
kN	KiloNewton

SINTESIS PERANCAH KARBONAT APATIT/GELATIN MELALUI KAEDAH PENGERINGAN DAN PEMBEKUAN

ABSTRAK

Dalam kajian ini, poros karbonat apatit/gelatin telah berjaya dihasilkan. Sintesis serbuk karbonat apatit telah berjaya dihasilkan melalui proses campuran asid fosforik dan kalsium hidroksida. Spektroskopi inframerah transformasi fourier, mikroskop elektron pengimbas, difraksi sinaran-x, analisis saiz zarah dan karbon hydrogen nitrogen menunjukkan sintesis serbuk karbonat apatit adalah asli. Selain itu, poros gelatin berliang juga dihasilkan melalui proses pengeringan dan pembekuan. Dalam kajian ini, poros gelatin berliang telah disintesis dengan menggunakan kepekatan ejen silang yang sama tetapi suhu yang berbeza iaitu -10 °C, -20 °C dan -40 °C. Keputusan kajian menunjukkan suhu yang rendah akan menaikkan ciri-ciri mekanikal perancah dari 1.01 MPa hingga 16.7 MPa. Kandungan gelatin dan carbonat apatit yang telah digunakan dalam kajian ini adalah 5g, 10g dan 15g. Selain itu, poros ini juga telah dikenakan dengan suhu yang berbeza. Kandungan gelatin yang tinggi akan menyebabkan ciri-ciri mekanikal bertambah. Ujian mampatan menunjukkan poros karbonat apatit/gelatin dengan suhu -40 °C telah mendapat kekuatan mekanikal yang paling tinggi iaitu 16.7 MPa manakala poros gelatin berliang pula mendapat 0.417 MPa. mikroskop elektron pengimbas menunjukkan saiz zarah yang besar akan melemahkan kekuatan poros tersebut..

SYNTHESIS OF POROUS CARBONATE APATITE/GELATINE VIA FREEZE DRYING METHOD

ABSTRACT

In this research, CO_3Ap/gelatine scaffolds were fabricated by freeze drying method. CO_3Ap powder were synthesized by reaction of phosphoric acid (H_3PO_4) and calcium hydroxide ($Ca(OH)_2$). FTIR, XRD, SEM, particle size analysis and CHN showed that the synthesized of CO_3Ap powder was pure CO_3Ap powder. Porous gelatine scaffolds was fabricated using freeze drying method. Three porous gelatine scaffolds were synthesized at different freezing temperature of -10 °C, -20 °C and -40 °C. As a result, lower freezing temperature increases the mechanical properties of porous gelatine scaffolds from 1.01 MPa to 16.7 MPa. 5 wt.%, 10 wt.% and 15 wt.% of gelatine content and CO_3Ap powder were used in this research to study the effects of the scaffolds. Besides, different freezing temperature of porous CO_3Ap/gelatine scaffolds also were been synthesized. Higher gelatine content will increase the mechanical properties of scaffolds. The highest mechanical strength for porous CO_3Ap/gelatine was 16.7 MPa with 15 wt.% of gelatine content at the temperature -40 °C while 0.417 MPa for porous gelatine scaffolds at the temperature -40 °C. SEM results showed the increased of particle size and pore size also will effects the mechanical behavior of the scaffolds.

CHAPTER 1

INTRODUCTION

1.1 Background

Regenerative medicine and tissue engineering are becoming more important in our modern medicine. These emerging fields usually involved advanced materials with rigid demands in terms of biocompatibility, biodegradability, mechanical strength, cell adhesion, proliferation and differentiation. Many ideal materials with all the requirements are generally not available. Bone fractures are very difficult to heal by themselves although within a couple of weeks. Besides, with the another condition of bones such as tumor resection or comminuted fractures will make the bones heal more slowly. This situation happen because of critical size bone defects (Dubruel & Vlierberghe, 2014a). The biomaterials will stabilizes the defects of bone and also will allows the bone growth (Dubruel & Vlierberghe, 2014a). The architecture of bone is complex, hierarchically constructed and has a function such as structural stability, protective environment for cells and also as a storage place for mineral ions and biomolecules. Therefore, the suitable implant materials for bone substitutes is very difficult and the materials must be successful simultaneous with the function and bone properties. For bone regeneration, the implant should be integrate into the surrounding of tissue that is called osseointegration, the implant also should promote the cell attachment, proliferation and migration of osteoblastic cell lines (osteoconductivity) and also can support the bone growth (Stevens et al., 2008).

There are four components that requires for bone regeneration that are morphogenetic signal, the responsive host cell that reacts with the signal, a suitable carrier of this signal that can transport to the specific sites and well vascularized host bed (Karen et al, 2000). Scaffolding materials can effects the formation of bone from the surrounding tissue. Besides, it also can act as a template and carrier for implanted bone cells. When the bone is regenerate, the implant should be degrade because to avoid the second surgery. Thusly, the degradation speed of the implant has to be same with the speed of newly formed bone. In other hand, if the implant degrades too fast and the bone is not good enough to perform, it will break again and fail (Dubruel & Vlierberghe, 2014a).

Biomedical and healthcare applications for biomaterials are consider the natural than food or drugs that usually used as a therapeutic or diagnostic system. Besides, it also used as a repair, support, regenerated and also to replace the defect of hard tissue and soft living tissues (Antoniac, 2016). The types of host tissue response can be divide into four generations. The first generation is to provide an acceptable combination of physical properties to match the replaced tissue. The second generation is to aim the encourage bonding to the host tissue and enhance the integration by promoting specific response from the host site and stimulating the growth of new tissue while the third generations is marked by a high degree of multidisciplinarity and interdisciplinarity such as materials in the science biology. The last generation is represented by the biologically of biomimetic and smart biomaterials. This generation provide new solutions to treat diseases, support tissue regeneration and also to rebuild body parts (Liu et al., 2002).

Polymers consist high molar mass chains that are usually composed of a large number same or different repeating units that are known as monomers. Monomers are the number of repeating units in a polymer that will show the degree of polymerization. In other hand, polymers have a very differences chemical structure and properties such as chemical composition, polymerization reaction type and based on the degradation and stability. The largest class of materials that recently used in a clinical application is a biomedical polymers because of it properties such as diversity and versatility (Antoniac, 2016). Polymer properties such as crystallinity, molecular-mass distribution and average molecular weight are the major properties that need to control during synthesize. Functional polymers are a special class where the polymer chain have a one or more reactive groups that will attached the end of the chain and also at the backbone. Functional polymers usually used for biomedical application because it functions as a building blocks to design complex structures such as biocompatible surface and scaffolds (Antoniac, 2016). Polymer are strongly approved safely to use inside human body because of it degradation properties.

In a tissue engineering, gelatine can be used as a scaffolding material because it is a natural polymer that extracted from collagen (Choi et al., 2001). Besides, the crosslinking of gelatine-based are very efficiency for a wound dressing (Hong et al., 2001). The properties of natural polymer are biocompatibility, biodegradability, low toxicity and cell signalling while the synthetic polymers have a properties such as chemical stability, thermal stability and mechanical properties. Besides, natural polymer can improved the cell viability and tissue growth while the syhthetic polymer are easy to fabricate although in different shapes. Common polymer for medical composite are presented in table 1. Furthermore, natural polymer are very potential to use in

biomedical applications because the properties are very biocompatibility and biodegradability. Next, the natural polymers possess many functional groups that available for further chemical and enzymatic modification. This natural polymer can be biomaterial because it allows an enormous variety of biomaterials that have same properties with another molecules (Yannas, 2004).

To overcome this problem, composite materials such as carbonate apatite (CO_3Ap) powder are used by combining the specification of its different constituents (Dubruel & Vlierberghe, 2014b). Bone apatite is the carbonate apatite that contains 6-9 mass% of carbonate in its apatite structure are not good enough if want to compared with hydroxyapatite. The hydroxyapatite can be sintered and also can make the hydroxyapatite free from carbonate. Carbonate apatite cannot be sinter because it has thermal decomposition at high temperature when sintering. Based on the dissolution-precipitation reaction methodology, it shows the composition of the precursor turns to the carbonate apatite. Besides, carbonate apatite also has a higher and good properties of osteoconductivity than sintering the hydroxyapatite. Furthermore, the most interesting of carbonate apatite is carbonate apatite will replace the bone when implanted in the bone defect while the sintering of hydroxyapatite will remain in the bone defects and stay in the original shape. This reasons shows that carbonate apatite is the most suitable bone replacement for next generations (Antoniac, 2016).

Table 1.1: Common polymers for medical composites, applications and several related commercial product (Antoniac, 2014)

Natural Polymers		
Polymer	Biomedical applications	Commercial Product
Collagen	Hemostatic scalant; wound, healing, skin and bone graft substitute	Integra, Helistat/ Integra LifeScience; FloSeal
Chitosan	Wound dressing, dentistry	HemCon dressing/ Hemcom Medical Technologies
Alginate	Wound healing dressing	AlgiDERM/ Bard Medical Division; Algisite/ Smith and Nephew; Hyperion Advanced Alginate Dressing/ Hyperion Medical; Kaltostat/ ConvaTec; Tegaderm/ 3 M Health Care; Kalginate/ DeRoyal; Curasorb/ Kendall; Maxorb/ Medline
Biostable synthetic polymers		
Polyamide	Nonabsorbable surgical suture; wound dressing	Kevlar/ DuPont; Ethilon/ Ethicon Inc; BioBrane/ Bertek Pharmaceutical Inc
Biodegradable synthetic polymers		
Poly(vinyl alcohol) (PVA)	Cartilage replacement	Cartiva SCI/ Cartiva, Inc.

1.2 Problem Statement

Nowadays, synthetic bone apatite is widely used as a substitute material for filling bone defects as it possess good biocompatibility and osteoconductivity. Osteoconductivity is a characteristic whereby the graft acts as a permanent and resorbable scaffold, mechanically supporting ingrowth of vessels and new bone from the borders of the defect into and onto its surfaces (Oryan, et al., 2014). Since the carbonate apatite is the bone apatite, at the physiological condition, carbonate apatite will be the most stable phase thermodynamically. Therefore, carbonate apatite powder must be fabricate by using precipitation reaction method by using a suitable precursor (Matsuya et al., 2010). However, one major drawback of this biomaterials is their low mechanical strength that displays brittleness (Takamasa Onoki, 2011). Another approach to further improve porous CO_3Ap/gelatine scaffolds by increasing the gelatine content and CO_3Ap powder content. The biocompatible, biodegradable and non-immunogenic of gelatine will make gelatine very suitable for biomedical applications (Bigi et al., 2000). Besides, gelatine also have a good properties such as chemical degradation (Bigi et al., 2000). Although gelatine is easily soluble in solutions but it has poor mechanical properties and this poor mechanical properties can effects the gelatine when use as biomaterials. Different freezing temperature also another way to improve porous CO_3Ap/gelatine scaffolds. Besides, glutaraldehyde must be added in gelatine aqueous solution to improve the thermal and mechanical stability of the biopolymer (Bigi et al., 2000). Glutaraldehyde act as a crosslinking-agent to the gelatine in this research. Glutaraldehyde also has high efficiency of collagenous materials stabilization.

1.3 Objectives

The objectives of this research are:

a) To synthesize porous carbonate apatite (CO_3Ap) /gelatine scaffolds by using freeze drying method.
b) To investigate the effect of CO_3Ap/gelatine ratio on the properties of gelatine scaffolds.
c) To investigate the effect of temperature on pore formation of CO_3Ap/gelatine scaffolds.

1.4 Research approach

This work is divided into 4 parts which will be described in details in Chapter 3. Briefly, the first part is to synthesis the carbonate apatite (CO_3Ap) powder by using precipitation method. This method is the combination of H_3PO_4 and $Ca(OH)_2$. In this process, CO_2 gas must be flowen in the reaction because it will produce carbonate (CO_3). Temperature for this process is 40 ˚C. Then, the solution will be filtered by using filter paper for a 24 hours and put in the oven at the temperature 60 ˚C for 24 hours. In this research, gelatine will be immersed in distilled at 60 ˚C until swollen. The solution will be stirred at 350 rpm using magnetic stirrer to form a homogeneous gelatine solution. Glutaraldehyde will be added as a crosslinking agent to the gelatine. The gelatine solution will then be put in the mould and freeze for 24 hours. Next is the fabrication of porous CO_3Ap/gelatine scaffold by using freeze-drying method. The gelatine will be soluble in the distilled water at the temperature 60 ˚C and will be stirred using magnetic stirrer. After that, the solution will be put in the room temperature and be stir under the vigorous stirring at 300 rpm for a 15 minutes. Next, poly(vinyl alcohol)

(PVA) and CO_3Ap powder will be put in this solution. After that, the solution will be put in the desiccator for 10 minutes to reduce bubbles. Glutaraldehyde will be put in the solution for a one minutes under vigorous stirring but different speed that is 200 rpm because want to mix it for a second time. Next, the solution will be put in the freezer at different freezing temperature of -10 ˚C, -20 ˚C and -40 ˚C for 5 hours and will go through freeze-drying for 24 hours. The characterization and analytical techniques used to determine the properties of the porous CO_3Ap/gelatine scaffolds such as Scanning electron machine (SEM), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Particle size analyser, carbon hydrogen nitrogen analysis and compression test.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Biomaterials are materials that recently used in therapeutic or diagnostic system that is in contact with the tissue or other biological fluids. Metals, ceramics, carbon, glasses, synthetic polymers and composites are the examples of categories in biomaterials. Being utilized in the manufactured of various medical devices and pharmaceutical preparations, biomaterials also provide many solutions concerning medical fields and healthcare problems (Boccaccini & Cough, 2007) . Advances in medicine have changed the ancients concept of surgery to transplantation then proceed with regenerative medicine. Most approaches currently pursue are dependent on the ability to synthesize materials into 2D and 3D forms to achieve the desired clinical responses. In this aspect, the biodegradable polymeric biomaterials offer the advantages of being able to be eliminated from the body after fulfilling the intended purpose. Biodegradable polymers will become biomaterials of choices in tissue engineering and drug delivery areas because it play a pivotal role in reparative and regenerative medicine in treating the damaged or diseased tissue (Ma et al., 2004).

2.2 Bone

2.2.1 Overview of Scaffold requirements for bone generation

Bone is a specialized connective tissue that formed by cells and extracellular matrix (ECM). The essentials elements needed for bone regeneration are osteoinduction, osteoconduction and osteointegration (Engel et al., 2009). Regenerating bone remain to be an actual challenge In the field of biomaterials as its function in scaffolding become extremely important in the aspect of biocompatible and permeability for oxygen. Another main function of regenerating bone is, it will permit degradation process in concert with the formation of the new bone tissue (Kakar et al., 2006). The scaffold for bone regeneration has some structural and functional to the requirements as a 3-D network with appropriate chemistry and dimensional features such as microporosity and macroporosity. The interconnectivity of scaffold for bone regeneration is responsible for mechanical behaviour, stability and controllable degradation. In addition, bone grafts should be sterilizable and radiographically detectable. Able to be fabricated in geometrical shapes and cost effective is one of the characteristic of bone grafting should possess (Giannoudis et al., 2005). Moreover, specific characteristics for bone grafts such as the presence of biological cues, reproduction of morphological and structural properties are discriminate to the capacity of each biomaterial to suit more specific task for example in the induction of angiogenesis, osteoinduction and biomineralization.

Scaffold architecture plays an important role in determining the rate and degree of bone in growth. The pore size and pore volume in the microstructure of three-dimensional scaffolds have mainly effects on the secretion of extracellular matrices (ECMs) and cell growth (Akay et al., 2004). In this analysis, pore size was the main parameter that has been studied in this research. Besides, pore sizes of the scaffold also will effects the cell

binding, ingrowth and migration. Although large pores is very effective to the nutrient supply, metabolic waste removal and gas diffusion, but it also can lead to the low cell attachment and intracellular signalling (Oh et al., 2007).

2.2.2 Current Problem associated with bone grafting

Autografted bone remains the gold standard technique for augmenting bone regeneration. However, this technique is significantly limited by the availability of tissue and the need for a second surgical site. A number of allograft strategies have been developed as the result of the limitation associated with substitution bone. Demineralized bone substitute is the most common allograft materials and has now been commercially processed into a number of different forms for applications such as a structural graft. These products will promote osteoinductive nature of the biomaterials.

The main problem for bone grafting is for the tissue to be remain inert. When this process is failed to happened, the grafted bone fail to revitalize and will cause osteonecrosis. Plus, it will have poor osseointegration and eventually loss in structural integrity of the bone graft (Benevenia et al., 2000). Contributing to this failure, the injuries are requiring grafting to the augment bone regeneration. It also often occurs in the areas where there is compromised vasculature. A fully vascularized bone autograft such as tissue explanted from the fibula, has now been used with some success to address these failures.

2.2.3 Critical Issue in Tissue Engineering

A common feature of the most frequently used methods for fabrication of porous scaffold is the low level of reproducibility of the porous morphology. This issue poses evident difficulties and constraint when a particular scaffold aims at being validated for clinical applications. Most processing methods do not enable specifying the size, shape or the spatial distribution of the porosity. Frequently, many methods allowing to obtain towards level of interconnectivity of porosity. However, the eventual inhomogeneous distribution of the interconnectivity implies that some parts of the scaffold will not be able to be populated by cells. Furthermore, the lower the interconnectivity will cause difficulty in the local diffusion of the aqueous phase and hinders the hydrolysis of the biomaterials. It also will results uneven degradation of kinetics (Motta & Migliaresi, 2014).

All the amount, size and level of interconnectivity of the porosity are interrelated yet have the different impact on the quality of scaffolds processed. The amount of the porosity is inversely related to the mechanical properties. High levels of porosity are desirable by maximizing the opportunities for the generation of cells and enhanced tissue development. However, those high levels of porosity imply lower mechanical stability of the scaffold. The size of the porosity is a critical issue in the performance of the scaffold with respect to the cell migration. If the pores are too small, it may hinder the regeneration of the internal structure by cells (Motta & Migliaresi, 2014).

In summary, all aspects that are related to the porosity of the scaffold that may contributing to obtain enhanced performance or may lead to inconsistency and anisotropy of the properties of the scaffold. Thus, it is highly desirable to select the most appropriate biomaterials and production method. Besides, it also to further validate in detailed experiments that related to the specific cells on optimized pore structure to maximize the performance of the scaffolds.

2.2.4 Polymer as scaffolding material for bone repair

Different materials have been studied as carriers and to provide support for bone regeneration such as ceramics, metals, polymers and composite (Cheung et al., 2007). Among these, polymers represent an extremely broad and versatile category of materials from the point of view of its chemistry, structure, degradation behaviour, mechanical properties and processing methods. Plus, it also used in coating. Depending on the target application, different characteristics are preferred. Special effort was devoted to biodegradable polymer systems focusing in vivo degradation, bone restoration phenomena is programmed to be occur in consistent manner.

Protein and polysaccharide are natural macromolecules that used as a scaffold for bone repair due to their biocompatibility, biodegradability and importantly as they exhibit the same characteristic as ECM. On the other hand, synthetic polymers such as poly-a-hydroxyesters are extremely interesting to use due to the predictable biodegradability characteristics. A very interesting fact is when the reconstruction of large bone defects is aimed, the biodegradation becomes a controversial subject because it has poor

biomechanical properties (Dubruel & Vlierberghe, 2014a). Another key concern related to the used of some polymers in bone repair is the lack of biological cues inherent to promote desirable cell responses.

2.2.5 Polymeric Scaffolds for Tissue Engineering

In a tissue engineering strategy, cells are seed on a scaffold that acts as a template to guide cell growth and to facilitate the formation of functional new tissues and organs. Scaffolds promote new tissue formation by providing an appropriate surface and adequate spaces to foster and direct cellular attachment and migration. The design of a scaffold is critical because it affects the formation and ultimate function of tissues. There are many general well-accepted criteria for ideal scaffolds in tissue engineering applications although it could vary in some degree among tissues types (Ma et al., 2004). The characteristics of degradation can be effect by mechanical properties, bulk material and the surface of morphology. Generally, a tissue engineering scaffold should be biocompatible, biodegradable, porous, the surface must be conducive to the facilitate cellular function, mechanically stable for surgical handling and easy to manufacture. In addition, the scaffold also should have the ability to carry biological signals such as growth factors and to deliver in a controllable manner (Boccaccini & Cough, 2007).

2.3 Types of Porous Scaffold

2.3.1 Ceramic

2.3.1.1 Porous Ceramic

Porous ceramic has a great interest as scaffold for tissue engineering because its ability to bond with the host tissue. To be able to regenerate a tissue, a scaffold should act as a template for tissue to grow in three-dimensional. The template must be a network of large pores that are connected to each other. This will allow the essential nutrients to reach the whole network and stimulate blood vessels to grow into the pore network.

Ceramic slurries can be foamed to obtain a porous structure. The incorporation of bubbles is achieved by the injection of gas through the fluid. The gel casting method has been used to produced macroporous hydroxyapatite (HA) with interconnected pores. Besides, the compressive strength of HA foams also similar to the trabecular bone (Sepulveda et al., 2005). Porous HA also can be produced by hydrothermal transformation from reef-building corals. These methods employ the use of elevated temperatures, pressures and controlled atmospheres to convert the calcium carbonate skeleton into HA. The route has the benefits of preserving the original architecture since coral serves as a template to make a porous structure (Boccaccini & Cough, 2007).

2.3.1.2 Calcium phosphate ceramics

Calcium phosphates are the major constituents of bone mineral (Boccaccini & Cough, 2007). Table 2.1 shows the list of several calcium phosphates with it chemical formula and Ca/P ratio. The most extensively used synthetic calcium phosphate ceramic for bone replacement is hydroxyapatite (HA) due to the similarity in inorganic component

of bone and teeth. The stoichiometry of HA is highly significant where thermal processing of the material is required. Slight imbalances in the ratio of Ca/P can lead to the appearance of extra phases. If the Ca/P is lower than 1.67, β-tricalcium phosphate (β-TCP) and tetracalcium phosphate (TTCP) will be present with HA. If the Ca/P is higher than 1.67, calcium oxide (CaO) will be present with the HA phase (Boccaccini & Cough, 2007). TCP is a biodegradable bioceramic that is wet in media and can be replaced by bone during implantation. TCP has four polymorphs but the most commonly used are α and β.

2.3.1.3 Carbonate Substitute Apatite

Bone apatite is the carbonate apatite. By sintering the hydroxyapatite as a bone substitute cannot be success because hydroxyapatite is not resorbed by osteoclasts. Furthermore, carbonate apatite is thought to be superior to sintered hydroxyapatite as a bone substitute. Since the carbonate apatite cannot be sintered because of thermal decomposition at high temperature, the fabrication of carbonate apatite must being another method. The precipitation method is the best candidate to fabricate carbonate apatite. In this method, the difference between thermodynamically stability is use for compositional transformation. In other hand, all components of precursor or metastable phase will be dissolved partially in the solution followed by precipitation of the component precursor phase to form the final product. Besides, carbonate apatite has a stable phase thermodynamically than hydroxyapatite (Antoniac, 2016). Figure 1 shows the phase diagram of the solubility of hydroxyapatite and carbonate apatite as a function of pH. Figure 1 also shows carbonate apatite has a same composition with bone apatite.

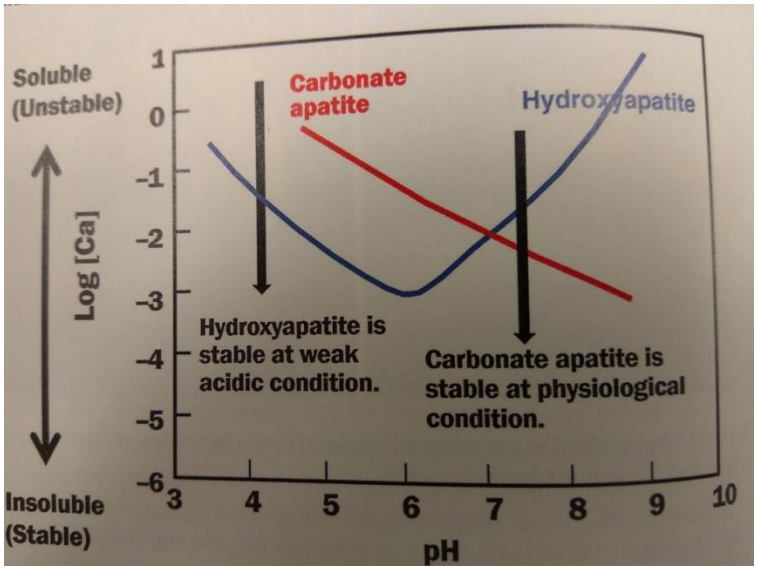

Figure 1.1: The phase diagram of the solubility of hydroxyapatite and carbonate apatite as a function of pH (Antoniac, 2016).

Hydroxyapatite in figure 1 shows lower solubility than carbonate apatite and this will make hydroxyapatite only can replace the limited function of bone apatite due to the difference in solubility in acidic region than carbonate apatite (Antoniac, 2016). This is the reason why carbonate apatite is more suitable to use in this research than hydroxyapatite.

Although stoichiometry of CHA has very low degradation rates but, it can be increased by substituting with other components that are found in biological apatites. The type of substitution will affect the rate of dissolution. Carbonate substitution contributes to the most soluble apatite (CHA), however, F substitution (for OH^-) will decrease the solubility to the lower than CHA. Furthermore, substitute apatite ceramics is interesting because it offers the potential to improve the bioactivity properties of implants.

Bone also is a carbonate substitute apatite with 5-8% CO_3^{2-} (Boccaccini & Cough, 2007). Therefore, the synthesis of a carbonate apatite may provide benefits over CHA.

Increasing the carbonate content is prove that can reduce the temperature at which decomposition occur to the phase of CaO and β-TCP (Boccaccini & Cough, 2007).

Table 2.1: Ca/P ratio of various calcium phosphates

Name	Abbreviation	Formula	Ca/P ratio
Tetracalcium phosphate	TTCP	$Ca_4O(PO_4)_2$	2.0
Hydroxyapatite	HA	$Ca_{10}(PO_4)(OH)_2$	1.67
Tricalcium phosphate	TCP	$Ca_3(PO_4)_2$	1.50
Calcium pyrophosphate	CPP	$Ca_2P_2O_7$	1.0
Calcium pyrophosphate dihydrate	CPPD	$Ca_2P_2O_7.2H_2O$	1.0

2.3.2 Polymer

2.3.2.1 Synthetic Polymers

Synthetic polymers represent the largest group of biodegradable polymers and this product can be produced under controlled conditions. In general, the synthetic polymers has mechanical properties such as tensile strength and elastic modulus (Gunatillake et al., 2006). Possibility risk such as toxicity, immunogenicity and favour of infections are lower with constituent monomeric units having a well-known or simple structure. Moreover, synthetic polymers provide the freedom to tailor the properties for specific applications. Some synthetic polymers are hydrolytically unstable and degrade in the body while others may remain essentially unchanged for the lifetime of the patient.

Biodegradable polymer is commonly used in repairing the nerves, skin, vascular system and bone (Antoniac, 2016).

2.3.2.2 Natural Polymers

In tissue engineering, scaffold materials play a significant role in directing functional tissue outcomes. The scaffold material should be selected based on the requirements of the tissue engineering study or application. Depending on which tissue is being engineered, some materials are more appropriate than others but in general, all scaffolding materials must satisfy the criteria such as purify, minimal adverse immunogenic response, biocompatibility, biodegradation and structural stability. Additionally, these materials must allow for porous scaffold assembly for cellular migration and proliferation, surface properties and chemical functionalization that promote desired cell functions, growth factor storage and release for controlling cell behaviour. Besides, the mechanical properties can mimic the structural characteristic of human tissue.

Natural polymers are well suited for tissue engineering application because it very biocompatible and can be restored. Compared to the synthetic polymers, natural polymers are advantageous for tissue engineering because it resembles the protein and polysaccharide extracellular matrix (ECM) components. Cells are recognized and possess the necessary binding domains for the amino acid and saccharide sequences. In contrast, the elastomeric backbone of synthetic polymers typically requires surfaces modifications or chemical functionalization in order to promote selective cell binding. Scaffold materials must be controllable and possesses a reproducible properties as it go

through the medical regulatory standards. Natural derived polymers structure and chemical components can vary widely between natural sources (Angele et al., 2004; Draget., 2005; Zeugolis et al., 2008). Table 2.2 shows the differences between natural polymers and synthetic polymers. For example of alginate, which is derived from marine algae and can have variable material properties depending on when it was harvested during the year due to changes in temperature and sunlight exposure as well as dependent on different species of algae (Draget et al., 2005). Last but not least, the establishment of tissue-engineered medical product standards will serve to guide the natural derived polymer production processes on meeting quality for clinical applications (Dornish et al., 2001).

Table 2.2: Differences between natural polymers and synthetic polymers (Antoniac, 2016)

Polymer for Tissue Engineering		
	Synthetically Derived	Natural Derived
Source of Raw Materials	Chemical Synthesis Plants	Animals Plants Microorganisms
Production Process	Polymerization Microbial Fermentation	Extraction
End Product	PGA PCL PEG	Collagen Fibrin Silk Alginate Starch

2.3.2.3 Gelatine

Gelatine is a derivative of collagen, where the hydrogen bonds between the triple helix α chains have been broken. Compared to collagen, gelatine has similar biocompatibility but less in the mechanical properties and the degradation process will be faster (Ratanavaraporn et al., 2006). Gelatine is highly soluble in aqueous solutions and forms thermally reversible hydrogels. Gelatine also can be process into a three-dimensional porous scaffold (Kang et al., 1999). Gelatine is often blended with other natural derived

polymer materials as a way to improve scaffold biological activity (Chang et al., 2003; Huang et al., 2005). Like collagen, gelatine is a crosslinked with glutaraldehyde. Crosslinking agent is needed as it will increase the mechanical stiffness level and prolong the degradation time (Huang et al., 2005). Regardless of crosslinking, gelatine has a low mechanical properties and faster degradation time. Gelatine that in sponges, powders and pastes are clinically used as hemostatic agents. For gelatine-based hemostatic gels, gelatine granules are combined with thrombin granules that will expand when applied to the bleeding surface. The expansion of the hemostatic agent helps to close the wound and will stop the bleeding. Depending on where the gelatine product is applied, complete resorption of material occurs between two days to the six weeks (Cappabianca et al., 2009). Besides, pharmaceutical industry now widely use gelatine as a biomedical field because it has hard and soft capsules, microspheres, wound dressing, sealants for vascular prostheses and adsorbent pad for surgical use (Bigi et al., 2000). In other hand, it is very easy to get and the price is cheap.

2.3.3 Composite

2.3.3.1 Composite Material Approach

A composite material consists of two or more chemically distinct phases such as metallic, ceramic or polymeric which are separated by an interface. The classification of engineering composite materials is based on the matrix materials or on the reinforcement dimensions and morphology (Boccaccini & Cough, 2007). Biodegradable composites for tissue engineering applications must exhibit specific properties such as high initial strength and tailored initial elastic modulus close to the elastic modulus of bone. In addition, it must have controlled the strength and modulus retention in vivo so

that it can provide the necessary support for cell attachment and proliferation as well as augment the tissue capacity to regenerate. Although polymers can be easily fabricated to form complex shapes and structures but a lack of bioactive function and being too weak to meet the mechanical demands in surgery. Thus, there are several reasons to combine the biodegradable polymers, bioactive ceramics and glasses for tissue engineering applications (Maquet et al., 2003; Rezwan et al., 2006).

Firstly, the combination of polymers and inorganic phase leads to the composite material with improved mechanical properties due to the inherent higher stiffness and the strength of the inorganic material. Secondly, the addition of bioactive phases to the bioresorbable polymers can alter the polymer degradation behaviour by suffering the pH of the nearby solution and hence the controlling of fast acidic degradation of the polymer. Incorporation of a bioactive phase in the polymer matrix helps to absorb water due to the internal interfaces formed between the polymer and the more hydrophilic bioactive phase. Hence, this providing a means of controlling the degradation kinetics of scaffold (Kim et al., 2005). Therefore, the development of composite materials for tissue engineering is attractive since their properties can be engineered to suit the mechanical and physiological demands of the host tissue by controlling the volume fraction, morphology and arrangement of the reinforcing phase (Rezwan et al., 2006).

Generally, two types of reinforcements are normally used for biomedical composites which are fibres and particulates (Ramakrishna et al., 2001). It has been shown that the increased volume fraction and higher surface area to volume ratio of inclusions favour bioactivity (Rezwan et al., 2006). Therefore, for a certain applications incorporation of

fibres is preferred instead of the particle (Kim et al., 2005). In addition, the mechanical properties are influenced by the reinforcement shape and size as well as by the distribution of the reinforcement in the matrix and the reinforcement–matrix interfacial bonding. The major factor affecting the mechanical properties and structural integrity of scaffolds are porosity. Pore volume, size, shape, orientation and connectivity will affect the porosity of the product.

2.3.3.2 Polymer/Apatite Composite Scaffold for Bone Regeneration

Polymer/apatite composite materials have been developed for mineralization tissue engineering application such as bone tissue engineering regeneration. Being similar to the major inorganic component of natural bone, the inorganic component such as hydroxyapatite (HA) in the composite scaffolds provides good osteoconductivity while the polymer component provides the continuous structure and design flexibility to achieve the high porosity and high surface area necessary for anchorage dependent cell such as bone cells to survive and differentiate. A well-developed material is produced by blending and phase separation techniques, polymer/hydroxyapatite composite that improved mechanical properties and osteoconductivity (Ma et al., 2001) The HA containing scaffolds improve osteoblastic cell seeding uniformity and show significantly enhanced expression of osteocalcin and bone sialoprotein over plain polymer scaffolds. Bone tissue formation throughout the scaffold has been demonstrated (Ma et al., 2001).