SCHOOL OF MATERIALS AND MINERAL RESOURCES ENGINEERING
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FABRICATION OF POROUS β-TCP SCAFFOLD 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 "**Fabrication of Porous β-TCP Scaffold 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 of this for any other examining body or university.

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LIST OF ABBREVIATIONS

Symbol	**Description**
β-TCP	Beta tricalcium phosphate
BTE	Bone tissue engineering
SEM	Scanning electron microscopy
EDX	Energy-dispersive x-ray spectroscopy
FTIR	Fourier transformed infrared spectroscopy
HA	Hydroxyapatite
G	α-L-guluronic acid
M	β-D-mannuronic acid

LIST OF SYMBOLS

Symbol	**Description**
wt%	Weight percent
β	Beta
α	Alpha
°C	Degree Celsius
Ɵ	Theta
mm	Millimetre
MPa	MegaPascal

FABRIKASI PERANCAH BERLIANG β-TCP MELALUI TEKNIK PENGERINGAN PEMBEKUAN

ABSTRAK

Beta-trikalsium fosfat (β-TCP) telah digunakan secara meluas untuk aplikasi bioperubatan. Terdapat beberapa kaedah untuk menghasilkan perancah berliang termasuk pemutus pelarut, fasa pemisah, prototaip pantas dan electrospinning. Dalam kajian ini, perancah β-TCP dan perancah β-TCP bersalut alginat telah dihasilkan melalui teknik pengeringan pembekuan. Buburan β-TCP telah dibekukan dan dikeringkan selama 24 jam diikuti dengan proses pensinteran untuk menghasilkan perancah β-TCP. Perancah β-TCP berliang terdiri daripada peratusan serbuk β-TCP yang berbeza (10 wt%, 20 wt% dan 30 wt%) dan telah dibekukan pada suhu takat beku yang berlainan (-10°C, -20°C, -40°C dan -80°C), untuk mengkaji kesan parameter ini pada pembentukan liang pada perancah. Imbasan mikroskop elektron (SEM) analisis untuk 30 wt% perancah β-TCP pada takat beku -10°C telah menunjukkan taburan saiz liang yang diterima dengan majoriti saiz berliang dalam julat 102.65 µm dan 372.90 µm. Tambahan pula, perancah ini juga mempunyai kekuatan mampatan tertinggi iaitu 0.31 MPa. Dalam usaha untuk mengkaji kesan salutan alginat pada sifat mekanikal, 30 wt% perancah β-TCP pada suhu -10°C telah dipilih untuk disalutkan bersama 5% natrium alginat. Selepas proses penyalutan selesai, spektroskopi inframerah transformasi fourier (FTIR) analisis dan analisis SEM telah dijalankan. Keputusan spektroskopi FTIR menunjukkan kewujudan ciri-ciri kumpulan fungsian natrium alginate manakala pemerhatian SEM menunjukkan permukaan yang lebih licin. Analisis ujian mampatan menunjukkan bahawa dengan lapisan natrium alginate pada perancah β-TCP telah meningkatkan nilai kekuatan mampatan kepada 0.8 MPa dari 0.31 MPa.

FABRICATION OF POROUS β-TCP SCAFFOLD VIA FREEZE DRYING METHOD

ABSTRACT

Beta-tricalcium phosphate (β-TCP) has been widely used for biomedical application. There are several methods to fabricate porous scaffolds including solvent casting, phase separation, rapid prototyping and electrospinning methods. In the present study, β-TCP scaffolds and alginate coated β-TCP scaffolds were fabricated using freeze drying method. β-TCP slurry was frozen and freeze dried for 24 hours followed by sintering process to form β-TCP scaffolds. The porous β-TCP scaffold were fabricated at different percentages of β-TCP powder used (10 wt%, 20 wt% and 30 wt%) and froze at different freezing temperature (-10°C, -20°C, -40°C and -80°C), in order to study the effect of these parameters on the pore formation of the scaffolds. Scanning electron microscopy (SEM) analysis for 30 wt% β-TCP scaffold frozen at -10°C revealed acceptable pore size distribution with majority pore size within the range of 102.65 μm to 372.90 μm. Furthermore, this scaffold also possessed highest compressive strength of 0.31 MPa. In order to investigate the effect of alginate coating on mechanical properties, 30 wt% β-TCP scaffold at freezing temperature of -10°C was selected to be impregnated with 5% of sodium alginate. After coating was completely done, Fourier transformed infrared spectroscopy (FTIR) analysis and SEM analysis were done. FTIR analysis identifies the existence of sodium alginate functional groups while SEM observation shows smoother surface. Compression test analysis shows that with the coating of sodium alginate on the β-TCP scaffold increased in the compression strength value to 0.8 MPa from 0.31 MPa were obtained.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Bone tissue is a living organ which mainly composed of both an organic and an inorganic component. Generally, bone consists about 10% to 20% percent water and approximately about 60% to 70% bone mineral. The remaining essential material existed in living bone is collagen with small trace amounts of proteins and inorganic salts. Typically, living bone is widely recognized organ that capable of forming highly functionalized connective tissue (Rodan, 1992).

There are several specific functions of bone in human body. The first basic function of this mineralized connective tissue is to serve as a framework to which other internal organs and soft tissues of the body are attached. Furthermore, bone acts as lever which is capable of adjusting significant power of the muscle and assist movement of the body (Clarke, 2008). Likewise, to ensure the bone receives enough supply of minerals that able to prevent breakage of bone, this connective tissue also serve as mineral reservoir for essential minerals such as calcium and phosphorus. All these functions demand for a balance and healthy bone system (Florencio-Silva et al., 2015).

Bone is a highly magnificent supporting framework of the body that has the capability to regenerate and remodel itself. Bone continuously undergoes healing as well as remodelling to replace the fracture bone and substitute it with new, well-built bone to help conserve bone strength (Fisher et al., 2016). Occasionally, there are millions of people from around the world suffer from this critical bone defects due to numerous basis including high energy injuries, tumor, bone loss, congenital defects and aging (Wiese & Pape, 2010). These deficiencies are gradually increasing and become one of the major clinical issues. Although, bone are capable to build itself by remodelling process however, severe defects requires assistance for bone regeneration (Yu et al., 2015).

Over the years, there are various type of different clinical alternatives used for treating bone defects including autologous and allogeneic transplantations using autografts and allografts. An autogenous graft involves transference of a tissue or organ from one part to another part of the body within an individual patient. It is widely recognize that autogenous bone graft are the golden standard therapy due to their ability to maintain the viability of cells and do not cause any immunological response in the patient (Amini et al., 2012). Simultaneously, allograft is a tissue transplantation technique which derived from donor body such as cadaver. Allograft provides several beneficial such as preserve crucial biologic and biomechanical features which helps to speed recovery (Whited et al. 2005). Despite numerous benefits, the disadvantages of allografts and autografts comprised of possibility of disease transmission such as human immunodeficiency virus (HIV) and Hepatitis, limited supply of donor tissue, complication to the donor site and formation of scar after surgery (Vang, 2006).

However, a noval approach to bone reconstruction has been widely discovered and developed. This innovative technique is known as bone tissue engineering. In 1993, Langer and Vacanti stated that tissue engineering is an interdisciplinary field which use the combination of particular aspects including cells, engineering principles and life sciences towards evolution of bone regeneration that are capable of restore and surpass better tissue function (Bleiziffer et al., 2007). Among the tissue engineering technique, a three dimensional (3D) scaffold offer temporary structural support during bone reconstruction for cell infiltration and act as a physical support to lead the cell activity into the targeted tissues or organs seems to be the most promising technique for the treatment of bone defects (Ikeda et al., 2014).

An ideal characteristic of scaffold for optimum bone tissue regeneration should possesses necessary design parameters in order to achieve outstanding rapid reproduction of cell with optimum attachment of cells. High porosity scaffold structure with interconnected pores are required for smooth nutrients transportation and provide site for cell to grow. In addition, it should have excellent mechanical properties to match with tissues at the implantation site (Izquierdo-Barba, 2014).

Generally, there are variety of different technique available to fabricate three-dimensional (3D) biomimetic scaffolds as reported in the previous studies such as polymer foam replication, gas foaming, solvent drying and particle leaching and freeze drying (Lu et al., 2013). Among these fabrication methods, freeze-drying method has been widely analyzed and demonstrated excellent control of pore size, distribution and homogeneity. Freeze drying method involved drying of pre-frozen wet slurries by the means of ice sublimation in vacuum. As the sample undergo drying stage, the ice crystals formed during freezing will create holes in the structure and fabricate final product of porous scaffolds. Prior to formation of pores with acceptable pore size and distribution, ice crystal size fast becoming the key component. Hence, the size of ice crystal can be controlled by adjusting both pre-freezing rate and pre-freezing temperature (Deville et al., 2006). It has commonly been assumed that the compressive strength properties of the porous scaffold is highly influenced by uniform pore distribution (Liebschner & Wettergreen, 2003).

Further analysis showed that for any type of materials used as bone substitutes, it is preferable that the porous scaffold to have sufficient mechanical strength not only to support new bone growth but also for ease of handling. In particular, porosity is inversely proportional to the mechanical strength of the scaffold. Thus significant increase in number of porosity leads to deterioration of the mechanical properties (Hadi et al., 2015). Therefore, in fabricating porous scaffold which sacrifices its mechanical strength, an alternative method to support the mechanical strength of the scaffold must be employed. Basically, the structure of human natural bone consists of both organic and inorganic part which supplies strength and partial elasticity to the bone. Therefore, the selected bone replacement material should be design with both organic and inorganic part. To achieve this, biodegradable polymers are often incorporated to form a composite scaffold to overcome the brittleness and difficulty of shaping and handling of ceramics scaffold while improving its mechanical properties (Sahari & Sapuan, 2011).

Selection of materials are becoming the chief principal for success implantation of scaffolds in bone tissue regeneration application due to the final features of scaffolds are greatly influenced by the materials properties. Significant potential of materials to slightly imitate the appearances and characteristic of extracellular matrix (ECM) has become the main significant features in selection of materials. Particularly, the suitable materials chosen for scaffolds can be divided into three sub-group: biopolymers, bioceramics and composite materials (Bazlov et al., 2016). Calcium phosphates materials which consist of hydroxyapatite (HA) and tricalcium phosphate (TCP) are broadly implemented in bone replacement practice (Amini et al., 2012).

Hydroxyapatite (HA) is a bioactive and bioresorbable calcium phosphate that forms the majority of the inorganic component of bone tissue. Although synthetic HA demonstrates good cytocompatibility, its usefulness as a scaffold material is limited due to its moderate to low solubility after implantation (Wiese & Pape, 2010). Therefore, Beta-tricalcium phosphate (β-TCP) has become the most promising materials for effective bone tissue regeneration due to several drawbacks of HA. However, there are certain drawbacks associated with the use of β-TCP scaffold. The main disadvantage of the β-TCP scaffold is that it has low compressive strength that displays brittleness. Therefore as a result, in this research, β-TCP powder with additional of polyvinyl alcohol (PVA), alginate and glutaraldehyde solution were explored in order to fabricate porous interconnected β-TCP scaffolds with good mechanical properties for load bearing application in bone tissue engineering (BTE) fields. The scaffolds were prepared using freeze drying technique under different freezing temperatures and different percentages of β-TCP powder. Lastly, in an attempt to improve the compressive strength, alginate is chosen to be used as the coating on the porous β-TCP scaffolds.

1.2 Problem statement

Engineered scaffolds are designed to function as a synthetic bone substituent which temporarily play the major responsibility in human bone. Specifically, these scaffolds are meant to act as framework which provide support structure to the surrounding bone tissue. Another significant roles of scaffolds include to act as adhesion sites for growth of bone cells and offer delivery vehicles for cell transportation (Fisher & Reddi, 2003). A key target for the microstructures of 3D scaffolds consist of high porosity which is approximately more than 90% and interconnected pore networks are desirable (Landi et al., 2003). Prior studies have noted that scaffold with higher percentage of porosity able to provide more structural space for cell attachment. Besides, pore interconnectivity also has been recognized as the factor found to be influencing the bone ingrowth during the initial bone replacement stage. One of the main reason is due to highly porous and interconnected porosity serve as a nutrient transportation. Besides, the pore size of the scaffold has also becoming one of the most rapidly declining factor which control the efficiency of cell activity and bone tissue remodelling (Martins et al., 2010).

Each types of cells has different favorable size of pores to permit highest attachment and rapid reproduction of cells (Boccaccini et al., 2009). Essentially, the recommended size of pores for fibroblasts cell are in between 5-15 μm while the suggested pore size for human skin tissue is within 20-125 μm. Last but not least, the most suitable pore size for bone tissue regeneration are approximately in between 100-350 μm. Consequently, successful cell infiltration and host tissue ingrowth developing methods for preparing scaffolds with suitable microstructures have become center of attraction among researcher. (Logeart-Avramoglou et al., 2005).

Previous studies have shown that the challenges in fabricating scaffold from bioceramics is to balance between the porosity of the biomaterial scaffold on one hand and maintaining its mechanical strength on the other hand. The empirical findings from previous studies have shown that there is an opposite relationship between porosity and mechanical properties of the scaffold whereas the strength tends to deteriorate as the value of porosity increases. In general, it seems that scaffolds should be designed to provide an interconnected pore network with enough percentage of porosity while maintaining sufficient strength and stiffness (Zohora et al., 2014).

Therefore, to fulfil the requirement of bone substitute with acceptable macro and microstructural characteristic, in the present study, a porous three dimensional (3D) β-TCP scaffold will be fabricated using freeze drying method. Throughout the entire study, critical parameters such as effect of different ratio between β-TCP powder and distilled water, effect of different freezing temperature on the pore formation and the effect of alginates will be investigated and the mechanical properties of the β-TCP scaffold will be evaluated.

1.3 Objectives

The main goal of this research is to fabricate three-dimensional (3D) porous β-TCP scaffold with high mechanical properties. The specific objectives of this study can be further listed as follow:

- To evaluate the effect of different ratio between β-TCP powder and distilled water on the pore formation of the ceramic scaffolds fabricated via freeze drying method.

- To evaluate the effect of freezing temperature on pore formation in freeze dried β-TCP scaffolds.

- To evaluate the effect of sodium alginate on the mechanical properties of porous β-TCP scaffolds.

1.4 Research approach

This research is divided into 4 parts which will be explained in details in Chapter 3. Briefly, the first stage involve preparation of β-TCP slurry. The preparation step is carried out by mixing deionized water, designed amount of β-TCP powder, 5 wt% of gelatin, 0.25 wt% of polyvinyl alcohol (PVA) and 0.25 wt% of glutaraldehyde solution. Then, the suspension is freeze in a freezer at different freezing temperature. The third part is the sintering of β-TCP scaffold. The scaffolds are first sintered at 500°C for 2 hours and undergo second stage sintering at temperature of 1100°C for 6 hours. Afterwards, the sintered β-TCP scaffolds will be immersed into 5% of sodium alginate solution by dissolving the sodium alginate powder with distilled water. Vacuum pump is used to ensure full penetration of sodium alginate into the porous β-TCP scaffold. The alginate coated β-TCP scaffold is then dried in an oven to remove residual solvent. Finally, the β-TCP scaffold with and without alginate coating will be characterized.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

In this chapter, three dominant themes of the research: calcium phosphate materials, alginate and freeze drying method will be introduce and discussed. This chapter also discussed the general overview of scaffolds in bone tissue engineering applications and the basic design requirements needed to achieve scaffold with interconnected porosity and high mechanical strength. Besides, relevant explanation regarding properties and structure of Beta-tricalcium phosphate (β-TCP) scaffolds are also been explored here. Finally, although freeze drying method is the main fabrication technique implemented in this experimental work, the scope of this literature review broadens to include brief explanation regarding other type of methods to produce porous engineered scaffolds.

2.2 Overview of scaffold in bone tissue engineering

In most recent studies, bone illness are being identified as one of the serious injuries that can be caused by the deterioration of the bone structure which in turn contributes to the bone's vulnerability to fractures. Nowadays, bone defects have become a huge health care problem worldwide. Taken together, thus, a major clinical and socioeconomic need is focused on the bone regeneration because of increasing trend of aging population and social development. A scientific approach to tackle this bone defect issue can be interpreted as bone tissue engineering (Dragica et al., 2013).

Bone tissue engineering (BTE) involves the implementation of porous 3D scaffolds. Typically, it serves as a temporary template for cell to grow and attach. In addition, the effectiveness of bone regeneration can be accomplished through implication of bioactive bone growth factors and extracellular matrix (ECM), which in return capable to increase number of cells through cell proliferation process (Vo et al., 2012). Another important practical implication is that scaffold plays a significant major role in tissue engineering applications as it imitates the function of natural bone (Gao et al., 2014). In the history of bone regeneration, various engineering scaffolds that used in conjunction with stem cells and gene therapy strategies have been recognized as a key factor in excellent new bone formation. Besides, scaffolds are fast becoming a key instrument in fixing up the segmental defects in both small and large animal studies (Wang et al., 2014).

2.2.1 Current scaffold designs

Generally, within tissue engineering fields, one of the most important theme is design of scaffold. Bone tissue engineering scaffold seems to be the favorable resolving alternatives in treating tissue failure, as explained in earlier overview of scaffolds. Besides, in general, the biomechanical processes of bone replacement are complicated, therefore, the requirements for scaffold design are diverse. According to previous studies by Hutmacher, the necessary design parameters for accomplishing the cell function leads to bone development is included the capability of bone tissue engineering scaffold to coexistence with living tissues without causing any harm. Besides, appropriate surface characteristic is necessary to promote excellent attachment and growth of the cell tissue. In addition, ultimately scaffold with interconnected, large pore size and high porosity can provoke rapid cell growth and achieved smooth nutrients and waste transportation (Frohlich et al., 2008). Another significant requirement of scaffold is that it should be mechanically stable with adequate mechanical strength properties to provide shape stability for the tissue loss (Chan & Leong, 2008). Overall remarkable design requirements for optimal scaffold can be explained in details from Table 2.1 while in

Figure 2.1 shows the factors involved in the optimized design of scaffolds.

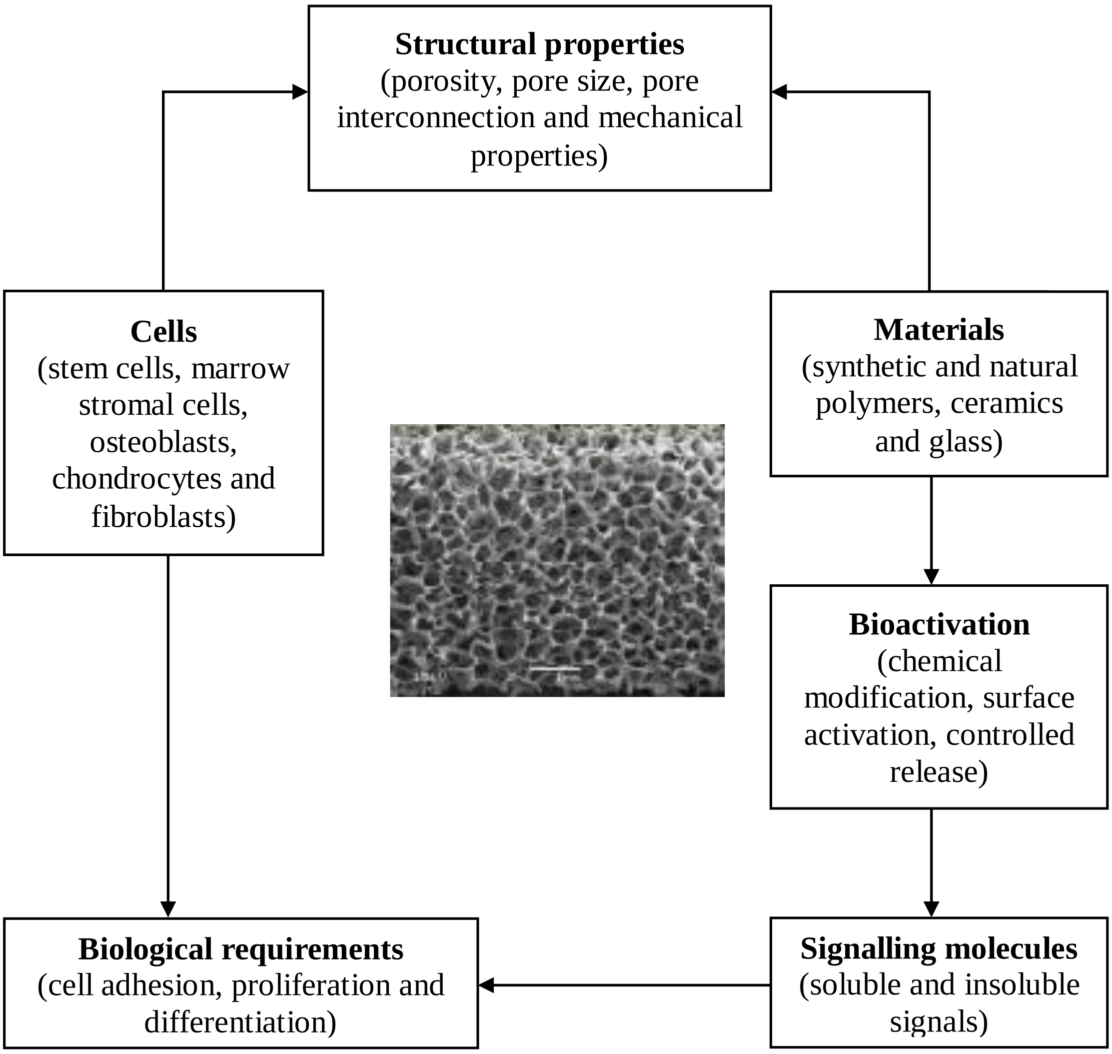


Figure 2.1: Schematic diagram of important key factors in developing a design for an optimal scaffold (Gerhardt & Boccaccini, 2010)

Table 2.1: Important scaffold design considerations (Velasco et al., 2015)

Design consideration	**Explanations**
1. Biocompatibility	Refer as the properties of scaffold materials which are biologically compatible with the living cell tissue and produce proper response in the recipient tissues.
2. Pore size and porosity	The scaffold should have large pore size accompanied with interconnected porosity to allow for attachment and growth of cell tissues. The scaffold required at least 100 µm size of pores while the accepted average percentage of porosity should be around 60%-70%.
3. Surface characteristic	The scaffold should have acceptable and appropriate surface properties as it play crucial roles in interaction of biomaterials including control of cell tissue growth after success attachment on the surface.
4. Mechanical properties	The scaffold should have adequate mechanical strength properties that match with surrounding tissue in order to prevent stress shielding from occurred which hinder the cell growth.
5. Biodegradability	The scaffold should have great ability to degrade within time either in vivo or in vitro surrounding environment as to create a site for growing of cell tissue.

2.3 Choice of biomaterials

In particular, due to various requirements in properties of scaffolds, this section only describes several available choices of biomaterials which widely used as a scaffold materials. American National Institute of Health provide the most acceptable brief definition of biomaterials. Biomaterials can be defined as any materials which capable to be used in longer period of time as a substitute substances for bone tissue failure (Bergmann & Stumpf, 2013). Three major biomaterials used in scaffold application include natural, ceramics and synthetic biomaterials. Nowadays, biomimetic natural biopolymer scaffolds have gained much attention to be used in tissue replacement applications as a bone tissue engineering scaffolds due to their excellent and faster body response towards infections compared to synthetic scaffolds (Brien, 2011). These natural biomaterials can be acquired from their natural sources and processed to produce scaffolds. The natural biomaterials are widely known to have various advantages ranging from their availability from renewable agricultural food resources, biodegradability to biocompatibility. Nevertheless, lot of previous researchers identified that natural biomaterials have several limitations. One of the major drawback associated with these natural biomaterials are limited mechanical strength and stability. Thus, it is not appropriate to be used in heavy load bearing applications.

The natural and synthetic biomaterials scaffolds are further categorized into different subgroups based on their sources. Consequently, natural biomaterials consist of subgroups such as protein-based biomaterials and polysaccharides based biomaterials. On the other hand, synthetic scaffold can be divided into polymer and ceramic biomaterials (Kohane & Langer, 2008). Figure 2.2 summarizes the classification of biomaterials for scaffolding.

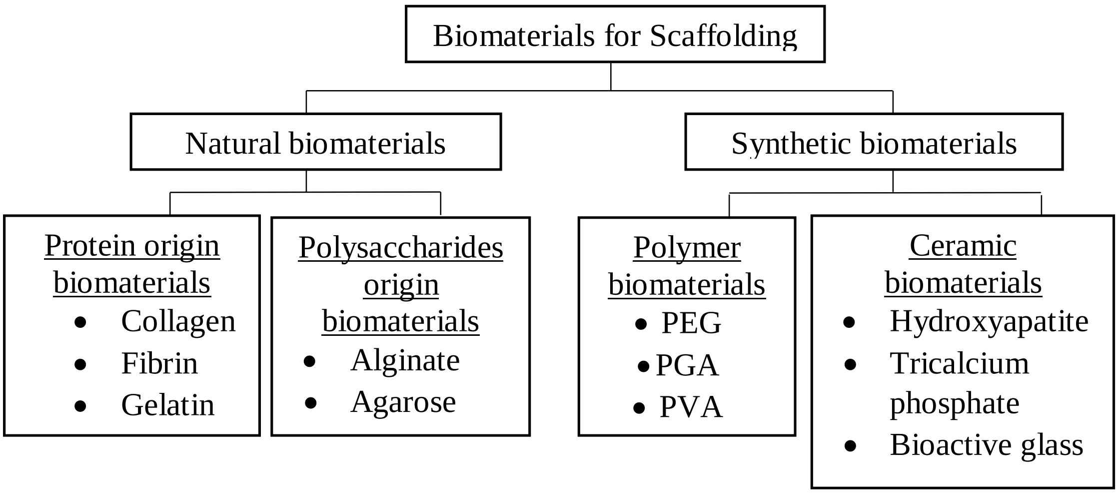


Figure 2.2: Classification of biomaterials for scaffolding (Steven, 2008)

2.3.1 Biopolymers

Basic polymer materials have drawn wide attention and been extensively used for bone tissue engineering application due to convenience control over biodegradability and ability to be processed (Lopes et al., 2012). Basically, polymer materials may be divided into two main sub-group. One is the synthetic polymer and the other is natural polymer (Asti & Gioglio, 2014). Natural polymers include collagen, silk fibroin, chitin and chitosan are commonly involved with tissue engineering principles and organ regeneration because of their superior ability to facilitate cell attachment. Likewise, a variety of synthetic biopolymers such as polylactic acid (PLA) and polyglycolic acid (PGA) are capable to provide numerous benefits to develop engineered scaffolds. The main advantage of synthetic polymers include having great potential to alter the mechanical strength properties in order to adapt with diverse applications. Additionally, biodegradable synthetic polymers also become appealing in tissue engineering field due to flexibility of forming into many kind of shapes along desirable pore size and interconnected porosity. Other than that, regeneration of tissue can be encourage by polymers with its chemical functional groups (Gunatillake et al., 2003).

2.3.2 Bioceramics

Commonly, bioceramics are favorable for its excellent corrosion and compression resistance as well as have good compatibility properties. Unfortunately, bioceramics materials has a number of serious drawback include poor mechanical properties which display brittleness and complicated to fabricate (Mamat et al., 2017). However, in contrast, polymers are mainly flexible and display an insufficient of mechanical strength and stiffness. Therefore, the composite materials with better mechanical properties can be produced by combination of polymer and ceramic materials which eventually improve both stiffness and strength of the engineered scaffolds (Dhandayuthapani et al., 2011).

The effectiveness of the composite materials has been exemplified in previous study for bone replacement applications. According to a research by Yoon et al. (2007), which carried out investigation on hydroxyapatite (HAP)/chitosan-alginate scaffolds by implementation of in situ precipitation method. The surface morphology, pore size and compressive strength of the scaffolds were analyzed. Collectively, these preliminary studies outline a critical role of HAP in improvising the mechanical properties of chitosan-alginate scaffolds (Gao et al. 2014). Hence, it is expected that ceramic-based composite materials have shown tremendous potential for bone tissue engineering application because of their adequate mechanical properties with highly porous microstructures (Hosoya et al. 2004).

2.4 Calcium Phosphate (CaPs)

In particular, example of the bioceramic material is calcium phosphate (CaPs). CaPs are being considered as the most favourable biomaterial for exogenous bone grafts in bone regeneration. Early in the 1920s, CaPs were first considered as filler for bone defect in clinical application and further incorporated in dentistry and orthopedics in the 1980s (Zheng, 2011). Recently, the use of CaPs as a bone replacement and repair is well recognized and obtained much attention as they have the essential characteristics which are chemically resemblance to bone mineral and capable to build up strong and cooperative interfaces with neighboring host bone tissues. Various forms of CaPs have been extensively studied for engineered scaffold research and are shown in Table 2.2 (Wang et al., 2014). Approximately, CaPs are divided into a sub-group of different compositions, considering the dissimilarity in each of their source of origin either natural or synthetic biomaterials as well as differences in fabrication methods. The porous scaffold can be constructed in a different physical form such as dense, microporous and macroporous (Nabil, 2013).

Table 2.2: Common bioceramic materals with respective Ca/P ratio
(Robert & Hans, 2015)

Ca/P	**Chemical formula**	**Chemical formula (IUPAC notation)**
0.5	$Ca(PO_3)_2$	Calcium metaphosphate
0.5	$Ca(H_2PO_4)_2.H_2O$	Calcium dihydrophosphate monohydrate
0.70	$Ca_7(P_5O_{16})_2$	Hepta calcium phosphate
1.0	$Ca_2P_2O_7$	Calcium pyrophosphate
1.0	$Ca_2P_2O_7.2H_2O$	Calcium pyrophosphate dehydrate
1.0	$CaHPO_4$	Calcium hydrogenphosphate anhydrate
1.0	$CaHPO_4.2H_2O$	Calcium hydrogenphosphate dehydrate
1.50	$Ca_3(PO_4)_2$	Tricalcium phosphate
1.67	$Ca_{10}(PO_4)_6\,(OH)_2$	Hydroxyapatite
2.0	$Ca_4O(PO_4)_2$	Tetracalcium phosphate

2.5 Beta-Tricalcium Phosphate (β-TCP)

2.5.1 Overview of β-TCP

Since the focus of this research is to fabricate porous β-TCP and it is known that β-TCP has the advantages of having superior balance control against rate of degradation and development of new bone tissues, therefore, extensive literature review has been made to understand the basic characteristic and properties of β-TCP. Basically, β-TCP may be divided into three main polymorphs according to their respective forming temperatures. First polymorph, beta-tricalcium phosphate (β-TCP), greatly stable at lower temperature than 1180°C till 1400°C. β-TCP is transformed into another type of polymorphs, either as an alpha-tricalcium phosphate (α-TCP) or super alpha-tricalcium phosphate (α'-TCP) depending on temperature value. α-TCP appeared at high temperature range within 1180°C-1400°C while α'-TCP phase observed at temperature above 1470°C (Prem et al., 2015). Once the phase transition had been occurred, the relative density value for calcium phosphate will slightly changes. The process of phase transformation involves gradual reduction in density upon treated with high temperature (Carrodeguas & Aza, 2011).

Fundamentally, the slow degradation rate of β-TCP has drawn numerous interest towards its significance usage in biomedical practices. In conjunction, β-TCP is publicly recognized to have sufficiently great biological performances which able to react well with other surrounding host tissues. Furthermore, β-TCP also has good ability to form direct bond with host tissue in order to fabricate new bone tissue replacement (Mehdikhani & Borhani, 2014).

2.5.2 Composition and structure

The crystal structure of β-TCP has been well characterized by neutron powder and x-ray diffraction (XRD) methods. Dickens et al. (1974) reported that β-TCP was a part from rhombohedral lattice systems and be rightly assigned as R3c space group (Kim et al., 2015). Table 2.3 displayed the physical dimension of unit cells in a crystal lattice of β-TCP, referred as a, b, c, α, β and γ as well as the number of formula units per cell (Z) along with cell volume (V), volume per formula unit (V_0) and theoretical density (Dth) (Carrodeguas & Aza, 2011). The Ca and PO_4 ions are the simplest repeating unit that exist in β-TCP. These unit cells are arranged along the [0 0 1] direction respectively as shown in Figure 2.3. Unfortunately, the crystal structure between α-TCP and β-TCP are different since the cation-cation (C-C) columns in β-TCP are absence. Alternately, the independent cation-anion (C-A) columns exist in β-TCP can be further divided into A columns and B columns. A columns containing two calcium atoms and one phosphorus atom in the arrangement of -P-Ca-Ca-P- while the slight deformed B columns comprise of six calcium atoms and four phosphorus atoms with series of chain of -P-Ca-Ca-Ca-P-P- as clarified in Figure 2.4 (Paul et al., 2015)

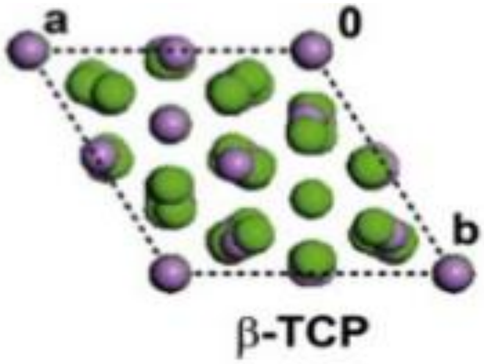


Figure 2.3: Schematic diagram of the projections of the β-TCP unit cells along the [0 0 1] direction: Ca^{2+}, green: P^{5+}, magenta: O^{2-} has not been represented for the sake of clarity (Carrodeguas & Aza, 2011)

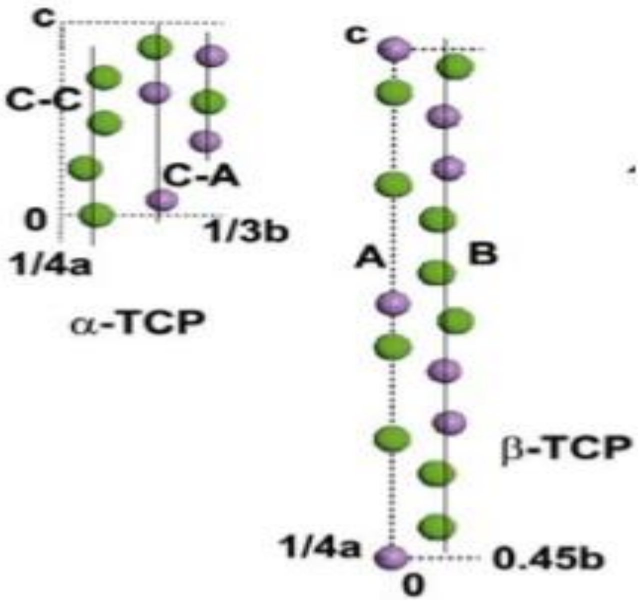


Figure 2.4: Fractional projections of the α-TCP and β-TCP unit cells on the bc plane showing the disposition of constituent atoms in columns oriented along direction [0 0 1]. Ca^{2+}, green: P^{5+}, magenta: O^{2-} has not been represented for the sake of clarity (Carrodeguas & Aza, 2011)

Table 2.3: Structural data of β-TCP (Carrodeguas & Aza, 2011)

Property	**Beta-tricalcium phosphate (β-$Ca_3(PO_4)_2$)**
Symmetry	Rhombohedral
Space group	R3c
a (mm)	1.0435 (2)
b (mm)	1.0435 (2)
c (mm)	3.7403 (5)
α (°)	90
β (°)	90
γ (°)	120
Z	21
V (nm^3)	3.5272 (2)
V_0	0.1660 (2)

2.5.3 Mechanical properties

In general, one of the most important criteria for successful implantation of scaffolds are the biomaterials should containing adequate mechanical strength. In addition, the significant mechanical properties of biomaterial is becoming a major area of interest within the field of BTE (Viswanath et al., 2008). Therefore, it is very crucial to maintain the mechanical properties of the scaffold structure after implantation step is done. Reconstruction and regeneration of soft bone tissue in load bearing application involve high mechanical strength. Ideally, in order for BTE materials to accomplish their goals in success implantation of scaffolds, it is very essential for the biomaterials to have adequate mechanical strength to withstand the force during cell growth. However, a major problem with β-TCP scaffold is that it has low mechanical strength and brittle (Dhandayuthapani et al., 2011).

Previous study has proven that addition of biodegradable organic polymer can aid to improve the mechanical strength of bioceramic scaffold. Among the available biodegradable natural polymer, alginate is known as a naturally occurring anionic polymer and has gained increasing attention to be the biodegradable polymers of choice to be incorporated in β-TCP scaffold. Miguel et al. (2015) have demonstrated that the development of alginate-calcium phosphate composite scaffolds with modified characteristic has excellent potential for successful bone tissue replacement. In his comprehensive analysis of this composite scaffold, Miguel et al. concluded that there is significant increase in mechanical testing analysis of powder-modified scaffolds with 2.5 wt% of alginate as compared to pure TCP (Castilho et al., 2015).

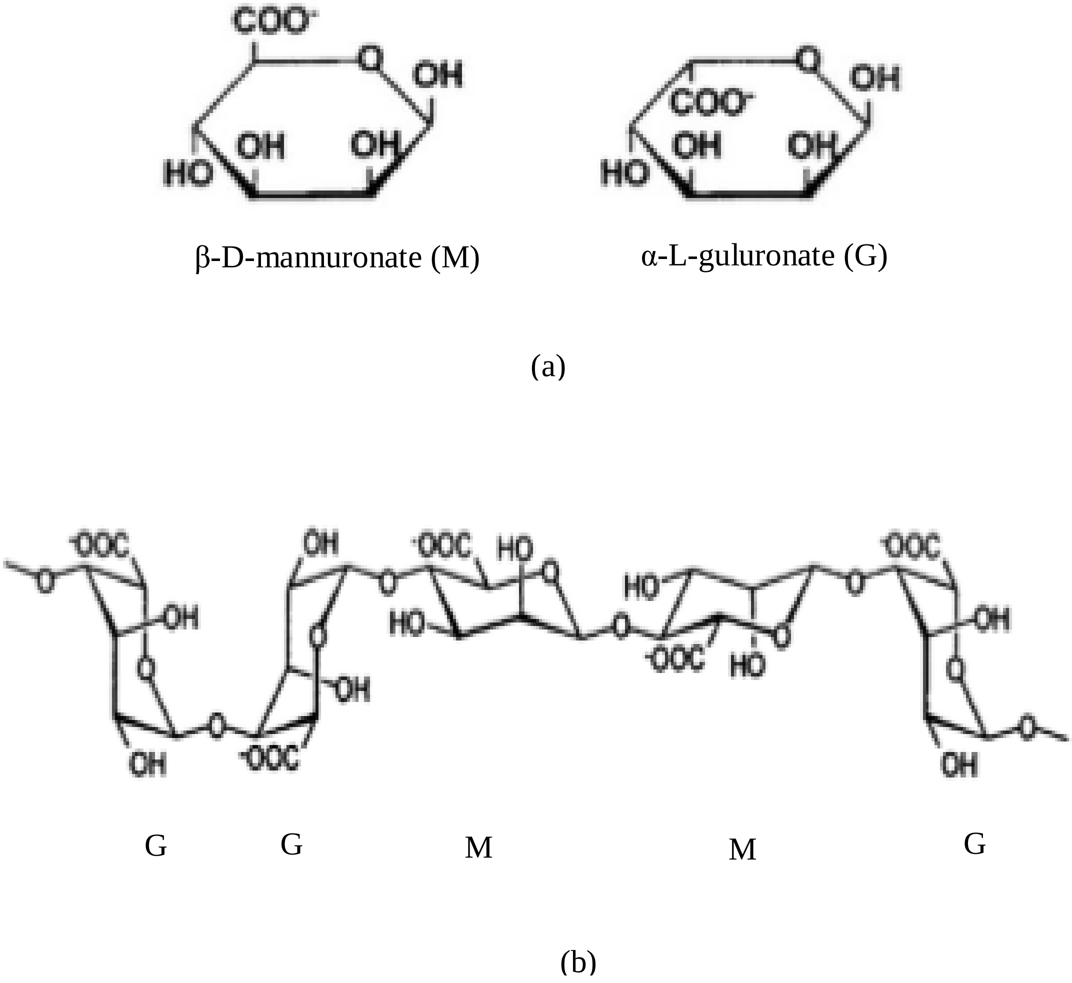
2.6 Alginate

More recent attention has focused on the provision of alginate in improvising the mechanical properties of biomaterials. Alginates can be loosely described as a family of naturally occurring anionic and hydrophilic polysaccharide. In this research, commercial alginate powder is used. Alginate is a biosynthesized materials that available in large quantities and typically comprises from 30 to 60% of brown algae (Mourino et al., 2010). The natural functionality of alginate is to give flexibility and mechanical strength to the seaweed. Additionally, it also serves as a water reservoir to prevent hydration of alginate once exposed to the air (Lee et al., 2012).

Interestingly, the quantity and quality of alginate in brown seaweed varies accordingly and depend upon the algae species, type and age of the tissues used for extraction of alginate, surrounding environmental conditions, season of harvest and extraction technique used (Latifi et al., 2015). In general, the commercial alginates are mainly derived from microorganisms such as Acophyllum nodosum, Lessonia nigrescens, Macrocystis pyrifera, Laminaria spp and Durvillea antartica. These alginates are widely used in food industry and biomedical applications in virtue of their thickening properties and ability to form a strong gel (Peter et al., 2012).

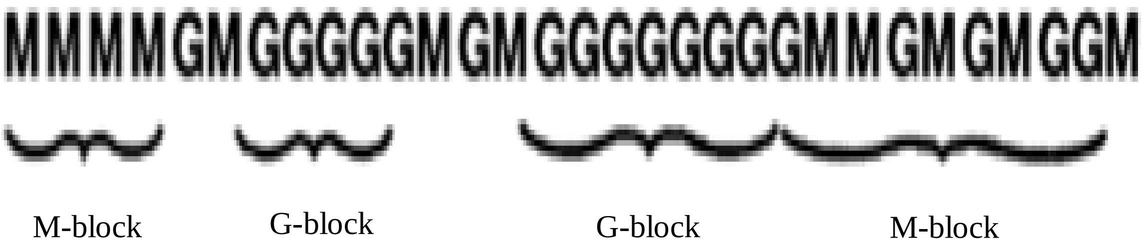
2.6.1 Structure and composition

Over the past decade most research in structure and composition of alginate has emphasized that the major component existed was D-mannuronate until recently, Fischer and Dorfel (1995) reported that L-guluronate content also appeared in the structure. Typically, alginates contain block copolymers based on the separation of ions between both manganese and calcium salts. However, it is to be noted that, the mannuronate (M):guluronate(G) ratio varies among the natural brown seaweed (Rhein-Knudsen et al., 2015). Ideally, the chemical characteristic of alginate is greatly influenced by the M:G ratio. Gels with dense and brittle features can be produced from alginate associated lower M:G ratio content. Meanwhile, alginate with higher M:G ratio content has the ability to produce more flexible gels. These findings have identified alginate as a complete unbranched binary copolymers family containing blocks of (1–4) linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) monomers (Aroguz et al., 2014). In particular, Figure 2.5 displays the structural characteristics of alginates which are made up of uninterrupted G residues (GGGGGG), continuous M residues (MMMMMM) and fluctuating M and G residues (GMGMGM) (Andriamanantoanina & Rinaudo, 2010).



β-D-mannuronate (M)

α-L-guluronate (G)



(c)

Figure 2.5: Structural characteristics of alginates: (a) alginate monomers; (b) chain conformation; and (c) block distribution (Draget et al., 2005)

2.6.2 Properties of Alginate

The most crucial characteristic of naturally occurring alginate is that it has ability to form ionic gels. Often, alginate incorporated in large network structure and ordinarily serve as gelling substances in extracellular matrix (ECM). A probable explanation of the gel-forming ability can be made based upon the “egg box model”. Conceptually, this model is a consequence of affinity towards cations and varies according to the composition and sequence of alginate chains (Braccini et al., 2001). According to previous reports, the G-residues in the G-block segments through the sequence of alginate chains will produce cavities that eventually ease high affinity towards cations primarily divalent cations as shown in Figure 2.6. Ions will bind at the GG symmetry site and causes dimerization of alginate chains. The gel network with dimeric junction zones forms after chemical reaction occurs between alginate chains (Myrnes, 2016).

A common cross linking agent in alginate salts such as sodium alginate has broad generality in biological systems, inexpensive and has great potential to build strong and rigid gels. Additionally, it is almost certain that alginate has highly viscous properties and