CHAPTER 2

Literature Review

In this chapter the theory and principles related to porous scaffold fabrication will be reviewed. The reviews will divided into 4 parts as follow:

1. Structure and properties of bone
2. Bone Tissue Engineering
3. Materials
4. Scaffold Fabrication Technique
5. Scaffold Characterization

2.1 Structure and properties of bone

In human bodies have approximately 20% of bone by mass. Bone are functional organ that not only to serve as a skeletal for human body but also act as an support and protection for soft tissue and act together with skeletal muscles to facilitate movement. Bone are rigid structure composed of calcified hard tissue and marrow [5]. Bone has a complex structure in which several level of organization from macro to micro scale. On a structural level two types of bone are identified: compact (cortical) and cancellous (trabecular) bone. Figure 2.1 shows bone structure of compact bone and cancellous bone.

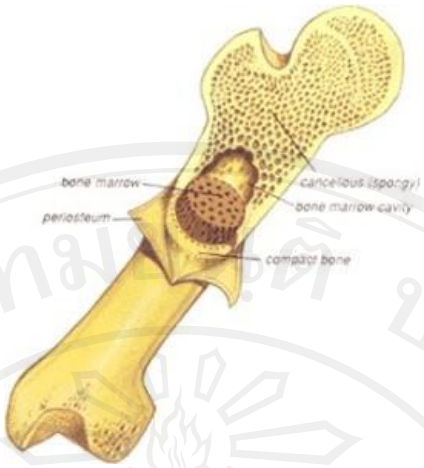

Figure 2.1 Bone Structure of compact bone and cancellous bone

Compact bone or Cortical bone is the dense form of the tissue. Which, can be found approximately 80% of the human skeleton. It is solid with 5-10% porosity and most found on the outer surface of all bones with 10-50 μm diameter pores [28] and compressive strength 100-150 MPa. The cortical bone can be separate into two groups, the first being long and short bones such as femur, tibia and ankle, the second being flat bone such as skull [6,7].

Cancellous bone or trabecular bone, makes up approximately 20% of the human skeleton. Normally found at the end of the long bones within the vertebrae, skull and pelvis. Trabecular bone have a high porosity (50-90%) with 300-600 μm diameter pores [28] and compressive strength 2-12 MPa so it can provide a lightweight structure to support joint [7, 8].

Bone matrix composition can be divide into 2 main types which is inorganic and organic.

The inorganic mineral part of bone consist of 65-70% of calcium phosphate in form of hydroxyapatite. While the main organic minerals part of bone are collagen. In addition, there are other organic materials such as glycoprotein, proteoglycan and sialoprotein which are involved with controlling growth and differentiating bone cells. Moreover, bone contains other component which are water, living cells and blood vessels. As a result of different composition, bone strength and toughness are varies depends on their composition. Minerals shows potential to stiffening bones and supporting compression,

while type I collagen support the tensile strength and toughness. Theirs mechanical behavior could be investigated by assess the whole bone *in vivo*.

2.2 Bone Tissue Engineering

To solve infection problem occurred regarding autograft bone tissue engineering has been introduce another technique for bone reparation.

Tissue engineering is an interdisciplinary field of research that applies the principles of engineering and the life science towards the development of biological substitutes that restore, maintain, or improve tissue function [24]. However, to bring the objective realistic knowledge from variety fields has to be assembled including the fields of physics, chemistry, engineering, biology, medicine and material science.

2.2.1 Bone Tissue Engineering Principles

Most tissue are composed of matrix and cells. Each matrix responsive for environment and structure influenced to cell ingrowths. Matrix consist of nutrients, water, cytokine and growth factors. For these reason, a template or a so called scaffold is needed to regenerate tissue. Scaffold will act as temporary matrix for cell proliferation until the target tissue completely regenerated while the scaffold gradually degraded. Suitable scaffold is the fundamental component for successful tissue engineering application. Material and the property of scaffold must be designed appropriately to produce the scaffold because it will affect cell survivability, bone growth and molecular signaling. The following parameters of the scaffold must be determined to ensure the scaffold property.

1. Biocompatibility
2. Porosity
3. Pore Size
4. Osteoconductivity
5. Biodegradability
6. Mechanical Property

These parameters will be described in the coming next topic scaffold characterization

2.2.2 Tissue Engineering Procedure

Tissue engineering procedure was shown in Figure 2.2

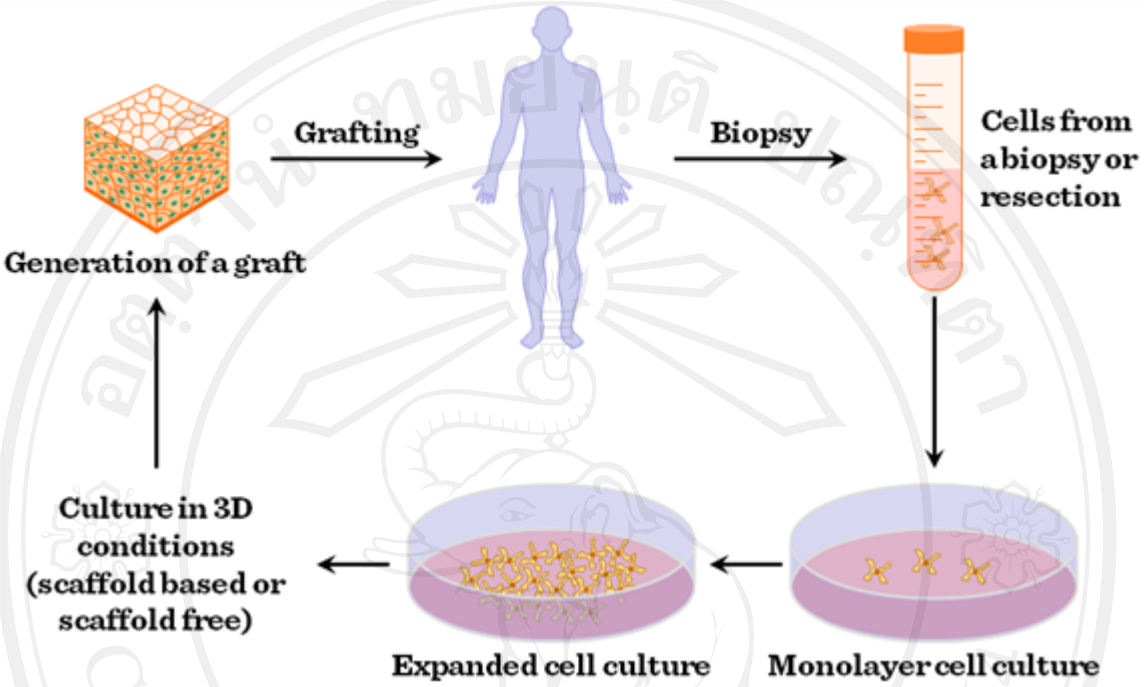

Figure 2.2 Tissue Engineering Procedure

Firstly, the matrices or scaffold are fabricated by biomaterials. Then the cell culture was performed until the cells is mature enough. Afterwards, the cells were seeded into the scaffold with the signaling molecule. Finally, the scaffold is implanted into the target defect area of the human body. After a while the bone will be successfully repaired.

2.3 Materials

This research aim is to fabricate the porous scaffold for bone tissue engineering. The selection of appropriate material to fabricate scaffold for tissue engineering application is important. For biomedical devices apart from biocompatibility, the ability to formed into complicated shapes are utmost important. Nowadays various materials have been used, including metal, ceramics, natural polymers, synthetic polymers. Biodegradable material were chosen for scaffold fabrication. Thus, Hydroxyapatite was selected to use as main

materials because they are able to extract from natural source and its properties are similar to bone. While, chitosan and fibroin were selected to blend with hydroxyapatite because of their suitable properties.

2.3.1 Hydroxyapatite

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is a calcium phosphate ceramic which has property similar to mammals bone minerals which can be synthesize from natural source such as coral, bovine bone or mollusk shell. Moreover, hydroxyapatite is a good candidate for bone tissue engineering application because of its bioactivity, osteoconductivity and biodegradability. Hydroxyapatite (HA) also has excellent compressive strength and its chemical composition and crystallographic are similar to inorganic component of natural bone. For this reason HA has been widely chosen to use in bone tissue engineering application. However, the weak point of HA is its brittleness, weak in term of tension and shear stress, and poor ability to form porous structure thus it need to blend with other polymer in order to enhance the properties. HA has been reported that it can withstand resorption in vivo the resorption rate was examined at 1-2% per year. In 2003 Saeri et al. synthesized HA from wet precipitation method by using calcium hydroxide as precursors [10] therefore HA used in this research wall be synthesized from mollusk shells by wet chemical precipitation method.

2.3.2 Silk Fibroin

There are 2 kinds of protein in silk of silk worm *Bombyx Mori* as shown in Figure 2.3 which are; hydrophobic glue-like serecin that bind the protein fiber together and hydrophilic fibroin which is a main structural center of the silk fibers with hydrophobic properties. Fibroin is an insoluble protein with beta sheets structure mainly consist of recurrent amino acid sequence Gly-Ser-Gly-Ala-Gly-Ala. Fibroin can be extracted from natural source such as silk worm cocoons, spider silk and red ant nests [11].

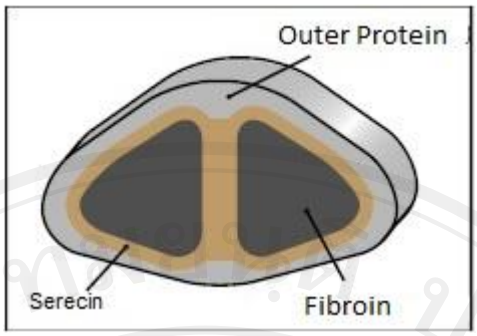

Figure 2.3 Structure of silk worm's silk

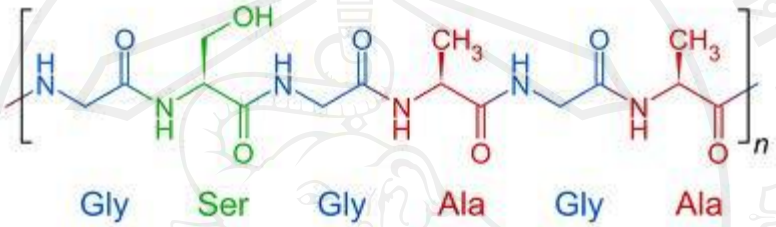

Figure 2.4 Primary Structure of Fibroin

There are many researches have proved that fibroin has good biological properties such as Biocompatibility, Biodegradability, water permeability and non-cytotoxicity. Moreover, fibroin was a good candidates for many tissue engineering application such as suture, scaffold, wound dressing and drug delivery system [4]. Moreover the result of Nandana et al. [3] showed that the presence of fibroin could stimulate the osteoconductivity properties of the scaffold. For these reasons, it was selected to use in this study because of its properties. But fibroin on its own have a weak point which is it's hard to form a porous structure so it needs to be blended with other polymer be able to form a porous structure.

2.3.3 Chitosan

Chitosan is a type of polysaccharide derived from chitin which is a copolymer of N-acetyl-D-glucosamine and D-glucosamine unit linked with β-(1-4) glycosidic bond with a structure similar to the glycosaminoglycans component of the extracellular matrix. Many forms of chitosan had been widely use in variety of application which include bone tissue engineering due to its good biocompatibility, biodegradability, non-toxicity, low cost [12],

ability to bind growth factor and Intrinsic antibacterial activity. Thus, chitosan were selected to blend with fibroin and HA because of its properties, ability to form highly porous structure and to reduce the brittleness caused by hydroxyapatite and fibroin. Moreover chitosan can promote the osteoconductivity because of its structure [13]. Normally, chitosan are insoluble in aqueous solution pH 7 [14] or above thus 2 wt% acetic acid solution will be used to dissolve the chitosan. From Nandana [3] works shown that chitosan blended scaffold showed good cell viability which can be a suitable candidates for tissue engineering application. However, chitosan is a derivatives of chitin so it can caused allergy in some case. In this research, the amount of fibroin will be fixed at the minimum amount that can be used to form the scaffold structure

2.4 Scaffold Fabrication Technique

There are many techniques to fabricate porous scaffold include solvent casting, freeze drying, polymeric foam replication, melt-based technologies and rapid prototyping technology, which were developed with the purpose of producing scaffold for tissue engineering application. These methods has their own advantages.

2.4.1 Solvent casting

Solvent casting technique is well known and widely use for scaffold preparation. This technique use mineral or organic particle in polymer solution and then use casting process to produce the scaffold. The advantages of this technique are controllable pore size and porosity while the disadvantages are limitation in interconnectivity pore connection and limitations of producing membranes up to 3 mm thick [15]. Moreover, the mechanical properties of scaffold fabricated by this method are weak when compare to trabecular bone.

2.4.2 Polymeric foam replication

This method can be performed by coating the polymeric foam with the structure slurry and let the foam set to dry at room temperature. Afterwards, the foam was burn out to become scaffold. This method can be used to create porous scaffold which are similar to trabecular bone. The advantages of this method is controllable size and shape of the pore in addition

to cells adhesion but this method is not suitable for naturals polymers that can be burnt out at high temperature.

2.4.3 Rapid prototyping technology

Rapid prototyping or solid freeform fabrication (SFF) can provide scaffolds with customizable shape and internal structure. The scaffold are formed by printing a concentrated suspension through a narrow nozzle to a substrate. Then the structure are heated slowly to bond the particle and decomposed the organic phase. This technique can be used to control porosity, pore size, pore distribution and create structure that improve tissue in growth in scaffold effectively.

2.4.4 Freeze-drying

Freeze drying method is the technique used for polymeric or bioceramic scaffold fabrication. Under the freezing process, ice grows in preferred direction and porous scaffold with the oriented microstructure have higher strength compared to a scaffold with randomly oriented structure the advantages of this method are highly porous structure, high pore interconnectivity and easy process but the disadvantages of this technique is small pore sizes and cost much time and energy [15]. However, from Yufeng et al. [16] studies results had shown that the pore size from freeze drying methods was still in acceptable range for bone cell proliferation.

There are some specific criteria required for fabricating scaffold (1) Fabrication method must not affect material properties in term of biocompatibility and chemical property (2) The fabrication techniques should accurately provide porosity, pore size, pore distribution and interconnectivity (3) Different scaffold obtained from the same techniques should present minimal variations in their properties. Thus, freeze drying methods was chosen to use to fabricate porous scaffold in this research due to its advantages. From Biman et al. [17] fabricated scaffold by freeze drying method by varying pre-freezing temperature and found that the pre-freezing temperature significantly affect the pore size and the porosity of the scaffolds. The less pre-freezing temperature showed the less pore size but more in

porosity. Thus, we can control the pre-freezing temperature to get the desired pore size of the scaffold.

2.5 Scaffold Characterization

Scaffold characterization will be analyzed in these following parameters.

2.5.1 Mechanical Properties

The mechanical properties of the scaffold is the one of the most important parameters because it also affect to the cell differentiation from Yu-Ru et al. [18] results shown that the stiffness of the scaffold affect to the cells differentiation and proliferation. The osteogenic properties are better on stiffer matrices compared to softer matrices. The methods widely used to evaluate mechanical properties are compressive strength test by using Universal Testing Machine to determined the behavior of materials under crushing load.

2.5.2 Biodegradation rate

Biodegradation is one of important properties of scaffolds. The good scaffold should have degradation rate approximate to the proliferation rate of the interested cell to support tissue regeneration. When the defect site is completely repaired the scaffold should be totally degraded. There are many ways to test the biodegradation rate such as soak the scaffold in PBS, SBF, lysozyme and observe the degradation rate. In 2011 Nandana et al. [3] had observed the degradation rate of chitosan/silk fibroin scaffold by lysozyme and PBS and found that the silk fibroin could prolong the degradability of chitosan scaffold.

2.5.3 Porosity and Pore Morphology

Porosity and Pore size also affect to the cell proliferation. The size of pore should close to the size of the cells to be able to penetrate into the porous structure of scaffold. The proper pore size for bone tissue engineering was well known to be within 200-500 μm range [19, 20]. Other requirement for bone scaffold is interconnective porosity that promotes the rapid bone ingrowth [17]. For these reason, scaffold should present sufficient micro-porosity. However, variations in porosity usually influence other scaffold properties including their

mechanical property. The common method to analyze the porosity of scaffold is liquid displacement method [4, 17] by using the liquid that can permeate into scaffold without shrinking or swelling the scaffold. Pore morphology of the scaffold will be analyzed by Scanning Electron Microscope (SEM) to show pore size and shape of the scaffold.

2.5.4 Biocompatibility

The biocompatibility must be determined to confirm that the materials will not disturb or induce any undesirable response on the host. The method of biocompatibility test are *in vivo* and *in vitro* studies. In vivo study is an experiment performed by using whole living organism as opposed a partial or dead mechanism. While, *in vitro* is experiment in controlled environment such as cell culture method. The cells used in these experiment include epithelial cells, mesenchymal stem cells, lymphocytes, fibroblasts and osteoblast. Hossein et al used peripheral blood mononuclear cells for compatibility test for hydroxyapatite particle [21] because of its suitability for assessing the immunoactivation and immunomodulation [22].

2.5.5 Swelling Property

The swelling test of scaffold determined the percentage of water absorption. Some materials able to absorb huge amounts of water or aqueous fluids in relatively short period of time [23]. Normally, natural polymer have high molecular weight and generally swell slowly to their equilibrium. Material with high swelling property would be form as sol-gel when swollen which is a good condition for ion and nutrient transport that widely use in drug delivery application.

2.5.6 Osteoconductivity

Osteoconductivity is the process which stem cells and osteoprogenitor cells are induced to move to a bone healing site and stimulate osteogenic differentiation pathway. However, for the large defect area, naturally osteoinduction may not sufficient. Therefore, the scaffold itself should have osteoconductive ability [5].