### **CHAPTER 5**

## **Conclusion and Suggestion**

This research describes the development of Fibroin-Chitosan-Hydroxyapatite scaffold fabricated by freeze drying method to use as bone filler. In the process, two kind of biomaterials were successfully synthesized: hydroxyapatite (HA) was synthesized from mollusk shell and silk fibroin was extracted from silk cocoon. HA and Fibroin was then used to fabricate the scaffold. Conclusion from this Fibroin-Chitosan-Hydroxyapatite scaffold research can be presented as follows.

#### **5.1 Materials Characterization**

HA and fibroin was synthesized in this research. While, HA was synthesized from mollusk shell. Silk fibroin was extracted from silk cocoon. From XRD and SDS-PAGE analysis proved that there are high chance that synthetic powder were HA and fibroin.

#### 5.2 Mechanical Property of Fibroin-Chitosan-Hydroxyapatite Scaffold

The compression test was performed to evaluate the mechanical property of scaffold following ASTM D395. The Young's modulus of porous scaffolds are in range 0.044-0.071 MPa which lower than human bone and cartilage. Thus, the porous scaffold in this study was properly used as bone filler at a non-load bearing location.

#### 5.3 Physical Property of Fibroin-Chitosan-Hydroxyapatite Scaffold

In order to produce the suitable scaffold, these following property must be taken into account: Porosity, Pore size, Swelling property and Compressive strength. All properties were surpass the minimum requirement considered viable to support bone ingrowth. Firstly,

the porosity of porous scaffold was found to be 93-95%, which is higher than the minimum requirement 90%. Second, the pore size of the scaffold was 200-400  $\mu$ m higher than the minimum trabecular bone pore size which was reported as 100  $\mu$ m and SEM images show that the scaffold has continuous structure and interconnected pore. Third, swelling property of porous scaffold showed the percentage of water absorption. The highest average swelling percentage of porous scaffold was 65.77% while the lowest was 41.59%. Finally, Compressive strength of porous scaffold was lower than the human bone cartilage. However, the porous scaffold can be use as bone filler at non-load bearing location.

#### 5.4 Biological Property of Fibroin-Chitosan-Hydroxyapatite Scaffold

Biodegradability was evaluated by PBS containing lysozyme. Degradation rate of porous scaffold was measured at 7 days. Scaffold with 100% fibroin has the highest degradation rate while scaffold with 100% HA has the lowest. This can conclude that increasing the amount of fibroin can accelerate the degradation of porous scaffold.

In this research, Peripheral blood mono nuclear cells (PBMC) were used to evaluate biocompatibility of porous scaffold. After seeded PBMC into the porous scaffold for 24 hours. XTT assay were performed in order to measured the optical density (OD) value. Then OD was used to calculate the cell viability percentage. More than 95% of cells can survive in the sample. This can proof that fibroin, chitosan and HA was non-toxic to living cells. However, there is a chance that chitosan or fibroin will cause allergic in some case therefore the allergenic test should be performed on the patient before usage.

In conclusion, from the result of all fibroin-chitosan-hydroxyapatite porous scaffold show that prepared scaffold using freeze drying method can be a promising candidates for bone tissue engineering in the future.

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#### Suggestion

The main point of this research focus on developing fibroin-chitosan-hydroxyapatite porous scaffold using freeze drying method and characterize the scaffold in physical and biological property for use in bone tissue engineering application. However, important relevant research remains. These are recommendation for future study:

- 1. Osteoblast cells are widely used in order to research *in vivo* study. Therefore using osteoblast cells in biocompatibility and osteoconductivity test for future work might be benefit to evaluate cell diferentiation and cell adhesion.
- 2. To study the of the ratio between Fibroin-Chitosan-Hydroxyapatite. In this research the ratio of fibroin and HA were varies. However, amount of chitosan can be affect to the property of the scaffold in some parameters. Therefore, in order to determine the appropriate condition for the scaffold the variation on amount of chitosan should be study in the future.

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