CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Three-dimensional porous scaffolds prepared from Thai silk fibroin were developed via freeze-drying and salt-leaching methods. Gelatin was used to incorporate into Thai silk fibroin using two various techniques. To obtain freeze-dried scaffolds, gelatin solution was blended with aqueous silk fibroin solution prior to freeze-drying. Type of gelatin suitable to incorporate with silk fibroin was evaluated. After that, porous scaffolds were treated by dehydrothermal and chemicals. Morphology of type A gelatin/silk fibroin scaffolds showed an uniform structure compared to type B gelatin/silk fibroin scaffolds due to the electrostatic force of both materials. As a result, type A gelatin was selected to incorporate with silk fibroin in this work and the effects of blending weight ratio and DHT treatment period on physical and biological properties of the freeze-dried scaffolds were investigated.

The different fabrication process resulted in a difference in the secondary structure of silk fibroin scaffolds. The structure of freeze-dried silk fibroin scaffolds was random coil while that of air-dried silk fibroin scaffolds after gelling was β-sheet.

Blending ratio was formed to affect the compressive modulus of the scaffolds. Scaffolds with high silk fibroin content (80-100wt%) possessed relatively high compressive modulus (~350 kPa). Swelling ability of gelatin/silk fibroin scaffolds tended to slightly decrease as increasing silk fibroin content due to the hydrophobic property of silk fibroin. There was no significant difference in the compressive modulus and swelling ability of each type of blended scaffolds when DHT treated for 24 and 48 h. *In vitro* culture showed that freeze-dried pure silk fibroin scaffolds tended to have a slightly more MSCs proliferated comparing to gelatin/silk fibroin and gelatin scaffolds. MC3T3-E1 cells culture indicated that the number of

proliferated cells decreased in all blended scaffolds as increasing silk fibroin content. This could due to the hydrophobic property of silk fibroin.

For the case of salt-leached silk fibroin scaffolds, gelatin was used to conjugate on the surface of silk fibroin scaffolds. Furthermore, the deposition of hydroxyapatite on silk fibroin and conjugated gelatin/silk fibroin scaffolds via alternate soaking in calcium and phosphate solutions was performed. After conjugating with gelatin, the structure of scaffolds were more fibrous with highly interconnection. The compressive modulus of conjugated gelatin/silk fibroin scaffold was higher than silk fibroin scaffold. In addition, hydroxyapatite growing on both scaffolds, with and without gelatin conjugating, resulted in less porous network and less interconnection as increasing the number of alternate soaking cycles. The hydroxyapatite deposition on the scaffolds was more upon the more cycles of alternate soaking resulting in higher compressive modulus but less swelling ability comparing to each initial scaffold without hydroxyapatite. Conjugated-gelatin/silk fibroin scaffolds showed a markedly increase in the number of MC3T3-E1 comparing to other scaffolds. In addition, the morphology of cells after 14 days culture of conjugated gelatin/silk fibroin scaffold indicated the extension of cytoplasm on surfaces. This revealed that gelatin conjugating was favorable to cell proliferation. Hydroxyapatite growing did not affect the number of proliferated cells. This was possibly due to the closed surface of scaffolds after hydroxapatite deposition.

6.2 Recommendations

Although the effects of gelatin type, blending composition, DHT treatment time, effect of fabrication method, and effect of hydroxyapatite growing on chemical, physical and biological properties of the gelatin/silk fibroin scaffolds have been investigated in this work, there are other interesting points which should be further considered as follows:

1. The detailed evaluation on the activity of proliferated MC3T3-E1, such as ALP activity, immunohistochemistry staining, on silk fibroin and conjugated gelatin/silk fibroin scaffolds should be performed to fully understand the biological characteristics of these scaffolds

2. Biodegradation property should be investigated in order to understand the degradation rate of silk fibroin scaffolds.

3. Further study on differentiation of mouse osteoblast-like MC3T3-E1 with induced medium should be explored in order to evaluate the potential of silk fibroin for bone tissue engineering application.