CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Crosslinked Thai silk fibroin/gelatin scaffolds were prepared by directly adding EDC/NHS in blended solution to stabilize gelatin in the desired scaffolds and subsequently fabricated via freeze-drying method. The effects of the weight blending ratios of Thai silk fibroin/gelatin, and chemical crosslinking were investigated. Morphology of Thai silk fibroin/gelatin scaffolds showed an uniform porous structure with smooth surface. The pore size of non-crosslinked Thai silk fibroin/gelatin scaffolds decreased as the weight blending ratio of Thai silk fibroin/gelatin was closed to 50/50. Crosslinked Thai silk fibroin/gelatin scaffolds also formed highly porous networks with smaller pore sizes. Weight loss (%) of crosslinked Thai silk fibroin/gelatin scaffolds was significantly lower than non-crosslinked Thai silk fibroin/gelatin scaffolds due to the crosslinking reaction by EDC/NHS. Also, weight loss (%) of non-crosslinked Thai silk fibroin/gelatin scaffolds was lowest in the case of the weight blending ratio of Thai silk fibroin/gelatin scaffold at 50/50 due to the electrostatic interactions of silk fibroin and gelatin, indicating a balanced charge of both materials. Crosslinked Thai silk fibroin/gelatin scaffolds significantly enhanced mechanical strength of scaffolds, comparing to non-crosslinked Thai silk fibroin/gelatin scaffolds in dry condition. However, the compressive modulus of non-crosslinked Thai silk fibroin/gelatin scaffolds was slightly higher than crosslinked Thai silk fibroin/gelatin scaffolds in wet condition due to great swelling ability of non-crosslinked scaffolds. *In vitro* biodegradability showed that the remaining weight (%) of scaffolds containing high amount of silk fibroin was higher than those with low silk fibroin content. In addition, chemical crosslinking by EDC/NHS could delay the biodegradability of crosslinked Thai silk fibroin/gelatin scaffolds. In term of the conformation changes, enzymatic degradation in collagenase solution and crosslinking reaction did not clearly affect the conformation changes of Thai silk fibroin/gelatin scaffolds. *In vitro* cell culture indicated that MSCs preferred to attach

and proliferate on Thai silk fibroin/gelatin scaffolds containing high amount of gelatin. This could be due to the RGD sequence contained in gelatin that was reported to promote cell adhesion and migration.

Furthermore, the addition of 70wt% hydroxyapatite particles in non-crosslinked Thai silk fibroin/gelatin 50/50 solution was prepared using homogenization method. Homogeneous distribution of hydroxyapatite granules in the scaffolds was observed. *In vitro* cell culture indicated that homogenized Thai silk fibroin/gelatin scaffolds with and without hydroxyapatite incorporation did not support proliferation. This might be the result of mass transfer limit in scaffolds. Another reason could be the toxicity of chloroform used during the homogenization method. The result of osteogenic differentiation suggested that osteoconductive potential of homogenized Thai silk fibroin/gelatin scaffolds with and without hydroxyapatite incorporation was similar. The highest ALP activity of MSCs cultured on both scaffolds was found at 7 days of osteogenic culture, indicating early osteoblastic differentiation stage.

It could be implied from this study that blended Thai silk fibroin/gelatin scaffolds could support *in vitro* cell culture, regardless of crosslinking and the weight blending ratio of Thai silk fibroin/gelatin. The preliminary study of homogenized Thai silk fibroin/gelatin scaffolds showed that these scaffolds could be used for osteogenic differentiation. However, further study should be performed to obtain suitable scaffolds for tissue engineering application.

5.2 Recommendations

For the case of homogenized Thai silk fibroin/gelatin scaffolds with and without hydroxyapatite incorporation, there are other interesting points which should be further investigated as follows:

1. The weight blending ratio of organic (Thai silk fibroin and gelatin) and inorganic (hydroxyapatite) should be investigated in order to obtain the suitable scaffolds as a biomaterial for tissue engineering.
2. Physical and biological properties, such as compressive modulus, *in vitro* biodegradability of homogenized Thai silk fibroin/gelatin scaffolds with and

without hydroxyapatite incorporation should be performed to fully understand the characteristics of these scaffolds.

3. Prior to *in vitro* cell culture, cytotoxicity test should be performed to screen the biocompatibility of these scaffolds.
4. The study on bioreactor for *in vitro* cell culture should be performed in order to improve mass transfer efficiency of scaffolds.