CHAPTER V
CONCLUSIONS AND RECOMENDATIONS

5.1 Conclusions

Polycaprolactone fibrous scaffolds were prepared by electrospinning process from a neat 12 % w/v PCL solution in 50:50 v/v DCM/DMF. The PCL electrospun fibrous scaffolds with a thickness of (130 ± 5) μm were obtained. The scaffold was subsequently immersed in various concentrations of 1,6-hexamethylene diamine (HMD) / isopropanol (IPA) solution (0.04, 0.06, 0.08, 0.10, 0.20, and 0.40 g/ml) for 2 hours at 30 °C. To determine the optimum concentration of HMD used in the step of aminolysis, ninhydrin method, degradation experiment, water retention experiment and SEM observation were used. The results show that the optimum condition for aminolysis are 0.20 g/ml HMD treatment for 2 hours at 30 °C. Density, porosity, and pore volume were evaluated to characterize the electrospun scaffolds. The NH_2 density on the aminolyzed PCL surface was confirmed and quantified using ninhydrin method. The NH_2 density increased with increasing 1,6-hexamethylenediamine concentration. However, when the concentration of 1,6-hexamethylenediamine is greater than 0.20 g/ml, the structural morphology of the electrospun PCL fiber scaffolds became worse. In the other word, water contact angle slightly decreased with increasing HMD concentration. This means that aminolysis can improve the hydrophilicity of the surface. Macromolecules i.e. gelatin type-A, gelatin type-B, bovine serum albumin, and crude bone protein were further immobilized using *N,N'*-disuccinimidyl carbonate (DSC) as a coupling agent. Various techniques; Attenuated Total Reflectance-Fourier Transform Infrared Spectrometer (ATR-FTIR), X-ray Photospectroscopy (XPS), Scaning Electron Microscopy (SEM), and water contact angle measurement were used to monitor the scaffold surfaces after each modification step. In XPS experiment, The N_{1s}/C_{1s} ratio was increased after immobilization with proteins (gelatin type-A, gelatin type-B, bovine serum albumin and crude bone protein) due to the large amount of nitrogen atom in protein structure was introduced. The potential use of the surface-modified PCL scaffolds as bone scaffolds was evaluated with a murine pre-osteoblastic cell

line (MC3T3-E1). The cytotoxicity test showed all types of proteins and PCL fibrous scaffolds released no substances at levels that were harmful to cells. The number of cells attached on these fibrous scaffolds was lower in comparison with that on TCPS at any given time point. There was the most viability of cells on crude bone protein-immobilized PCL fibrous scaffold among various types of the modified fibrous scaffolds. MC3T3-E1 proliferation was improved remarkably on the modified surface, with the cells growing on the bovine serum albumin-immobilized PCL fibrous scaffolds showing the greatest proliferation after cell culture as well as the highest ALP activity. In long term experiments, the image of scaffolds seeded with MC3T3-E1 for 21 days and stained with Alizarin Red-S and quantification of deposited minerals measured by UV-vis spectrometer confirmed that high intensity of stained minerals were observed on all type of immobilized PCL scaffolds. The CBP-immobilized PCL scaffold showed the highest mineral deposition compared with control, neat PCL, and other modified materials. This result supported that the crude bone protein immobilization was able to induce the cell differentiation to bone the most. All the obtained results suggested that bovine serum albumin and crude bone protein immobilization are an attractive method to fabricate of further developed fibrous scaffolds for bone tissue engineering.

5.2 Recommendations

Based on the present results, the following recommendations are suggested for future studies:

1. To study protein adsorption isotherm on all type of surface-modified PCL scaffolds.
2. To study long term use of surface-modified PCL scaffolds as bone scaffolds.
3. To study the potential use as bone scaffold in further developed fibrous scaffolds immobilized with bovine serum albumin and crude bone protein.