CHAPTER V
CONCLUSIONS AND RECOMMENDATIONS

In this thesis, polycaprolactone (PCL) films were prepared via solvent casting method. Chloroform, tetrahydrofuran (THF), acetone, 20:80 (v/v) EtOH:THF, 30:70 (v/v) EtOH:THF, and 40:60 (v/v) EtOH:THF were applied as solvents. Due to different solubility parameters of each solvent, the casted films had various surface topologies. Moreover, the surface of film casted from THF was treated with 1 M NaOH and 5 M NaOH for the surface modification by hydrolysis. In order to enhance the protein immobilization, the surfaces of PCL films were also successfully modified through a chemical process. In which, the amino groups were introduced by aminolysis of 1,6-hexamethylenediamine to the ester group on PCL surface. Bovine serum albumin (BSA), selected as a biomolecule, was immobilized on the modified PCL surface using *N.N'*-disuccinimidyl carbonate (DSC) as a coupling agent. The results from SEM and AFM indicated that the surface topography of the films was rougher with a higher difference between solubility parameter of the solvent and PCL. In which, the PCL film casted from 40:60 (v/v) EtOH:THF had the roughest surface with the average roughness of 0.97 μm while the most uniform film surface belonged to the film casted from chloroform with the average roughness of 15.64 nm. The result from water contact angle showed that the neat film casted from 40:60 (v/v) EtOH:THF was the most hydrophobic. However, after the protein immobilization, the film became more hydrophilic. The protein adsorption test indicated that the film casted from 40:60 EtOH:THF could adsorb significantly higher amount of protein and surface modification can even enhance the amount of protein adsorbed more than the neat film.

The biological evaluation of neat and surface-modified PCL film was demonstrated with a pre-osteoblastic cell line (MC3T3-E1). Indirect cytotoxicity test showed that all types of substrate did not release any toxic substance to the harmful level. Cell attachment and proliferation increased with culturing time. Cell attachment on most of the smooth protein-adsorbed substrates was lower than on the that of TCPS, except the rough protein-adsorbed substrate casted from 40:60 (v/v) EtOH:THF which showed a higher cell attachment and proliferation. Interestingly,

the greatest cell proliferation could be observed on the protein-immobilized substrate casted from 40:60 (v/v) EtOH:THF. Images from SEM represented cell morphology on the PCL films and can be used to confirm the result from cell attachment and proliferation test. It can be seen that cells were still round after 4 h of cell seeding and began to extend their cytoplasm after 24 h. The investigation on calcium deposition was obtained after the cell culturing for 21 days. The Alizarin Red-S staining showed that the highest intensity of stained minerals could be observed from the film casted from 40:60 (v/v) EtOH:THF. Despite a high cell attachment and proliferation on TCPS, it showed a low level of mineralization. Moreover, the protein-adsorbed PCL films exhibited a significantly higher level of calcium deposition than the neat PCL films of the same surface topology. It can be concluded that bovine serum albumin preferred to adsorb more on the rough surface and the presence of protein could help promoting cell attachment, proliferation, and differentiation of the MC3T3-E1 cells.

Recommendations for the future work would be to study other types of protein adsorption apart from BSA, such as collagen. Also, the investigation based on cell behavior and protein adsorption upon aligned versus non-aligned fibrous substrates is interesting.