CHAPTER V
CONCLUSIONS AND RECOMMENDATIONS

Polycaprolactone (PCL) film mats were prepared by solvent casting process. Amino groups were covalently introduced onto the PCL surface by the reaction between 1,6-hexamethylenediamine and the ester groups of PCL. The modified films were subsequently immobilized with biomacromolecules (i.e. crude bone protein or bovine serum albumin) by using *N,N'*-disuccinimidyl carbonate (DSC) as a coupling agent. To determine the presence of amino groups and hydrophilicity of the film mats after modification occurred, ninhydrin analysis method, water contact angle, ATR-FTIR and XPS were used. The results of ninhydrin showed that the free amino group density was influenced after the films were modified while XPS analysis confirmed the existence of crude bone protein and bovine serum albumin due to the higher N1s/C1s ratio obtained, that means, enriched with nitrogen element could be found in protein-immobilization. In addition, water contact angle showed the decrease of angle after surface of the film was modified, this can be concluded that hydrophilicity on the surface of materials become better after aminolysis and protein-immobilization occurred.

The potential use of the surface-modified PCL scaffolds as bone tissue engineering was evaluated with a murine pre-osteoblastic cell line (MC3T3-E1). The cytotoxicity test showed all types of proteins and PCL film mats released no substances at levels which were harmful to cells. The number of cells attached on these substrates were lower in comparison with that on TCPS at any given time point. While, MC3T3-E1 proliferation was improved remarkably on the bovine serum albumin-immobilized PCL, which showed the greatest proliferation on day 3 after cell culture. SEM images showed a morphology of the cells attached to TCPS and film mats. It was found that cells cultured on all types of materials were still on 6 h after cell seeding. Nevertheless, after 24 h of cell seeding, all of the investigated substrates showed an evidence of the extension of their cytoplasm. ALP activity of MC3T3-E1 grown on TCPS and film mats reached a maximal value on day 5 after cell culture. Among the various film mats investigated, bovine serum albumin-immobilized PLA showed the highest ALP activity at any given time. 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 modified PCL film mats. The bovine serum albumin-immobilized PCL scaffold showed the highest mineral deposition, followed by the crude bone protein-immobilized and aminolyzed PCL, respectively. All the obtained results showed that the protein immobilization is a good candidate to be used as a bone scaffold because it supported cell attachement, proliferation, differentiation and mineralization of osteoblast-like cells.

The recommendation of the future work is based on evaluation protein adsorption isoterm in each material which may effect on regulation of bone cell growth. Another recommendation may be required in designing and creating the suitable form of materials to investigate the *in vivo* of bone regeneration with implantation of such those materials.