CHAPTER VII
CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

In summary, 3D PCL scaffolds with highly porous and interconnected networks can be prepared using our modified solvent casting, particulate leaching, and polymer leaching techniques, which implement sodium chloride and PEG as porogens. SEM, visual slide microscopy and optical microscopy confirmed that the scaffolds fabricated using this method exhibit highly interconnected pore networks, equally distributed pores, and a relatively uniform pore size of 378-435 μm. The greatest porosity, pore volume, and pore size values and the highest interconnectivity were observed for the salt-PEG 1000 leached PCL scaffolds, further leading to the highest water absorption capacity of the materials tested. The potential for the use of these constructs in bone tissue engineering was evaluated in vitro using mouse calvaria-derived pre-osteoblastic cells (MC3T3-E1). An indirect cytotoxicity evaluation revealed that the scaffolds fabricated using our method released no substances at levels that were harmful to the cells. Moreover, SEM demonstrated that the majority of the cells seeded on the scaffold surfaces had expanded over the scaffold surface after 3 days, with the most expansion observed on the scaffolds generated by our method. The cells cultured on these new PCL scaffolds also yielded the highest mineral deposition values. Taken together, all of our results indicate the potential use of these constructs as bone scaffolds.

PCL/HA dual-leached scaffold has been prepared by combining solvent casting and salt particulate leaching with polymer leaching technique. In order to improve hydrophilicity of PCL/HA dual-leached scaffold, the PCL/HA was treated by alkaline. The PCL, PCL/HA, and NaOH treated PCL/HA dual-leached scaffolds have been extensively characterized in terms of thermal, physical and mechanical properties such as morphology, actual HA amount, compressive modulus, and water absorption capacity. The compressive modulus increased from ~ 58 kPa for the PCL scaffold to 258 kPa for PCL/HA dual-leached scaffolds. Mechanical properties of

PCL/HA dual-leached scaffold have been greatly improved by incorporating HA particles as fillers. Indirect cytotoxicity evaluation of these scaffolds with mouse fibroblastic cells (L929) and mouse calvaria-derived pre-osteoblastic cell (MC3T3-E1) indicated biocompatibility of these materials to both types of cell. The potential for use of these scaffolds as bone scaffolds was further assessed in vitro in terms of the attachment, the proliferation, the alkaline phosphatase (ALP) activity, and the mineralization of MC3T3-E1 that were seeded or cultured at different times. The obtained results showed that the both PCL and NaOH treated PCL/HA dual-leached scaffolds exhibited better adhesion of cells than the TCPS and the NaOH treated PCL/HA dual-leached scaffold exhibited better proliferation of cells than the corresponding TCPS and other scaffolds. Evidently, the cells that were cultured on all of the dual-leached scaffolds at 4 h appeared to be well-expanded and attach on scaffolds surface while that seeded on glass substrate was still in round shape. The cells that were cultured on NaOH treated PCL/HA dual-leached scaffold showed the most expansion on the surface of scaffold. In mineralization assessment of MC3T3-E1 on days 14 and 21, the most intensity of staining product for calcium deposition was observed on NaOH treated PCL/HA, followed by PCL/HA, PCL, and TCPS, respectively. Our results indicate that NaOH treated PCL/HA dual-leached scaffold possesses improvement in mechanical properties and hydrophilicity and its ability to support MC3T3-E1 cell attachment, proliferation, and mineralization for used as bone scaffolding material.

PCL, PCL-PHB, and PCL-PHBV dual-leached scaffolds have been prepared by combining solvent casting and salt particulate leaching with polymer leaching technique. The PCL, PCL-PHB, and PCL-PHBV dual-leached scaffolds have been extensively characterized in terms of physical and mechanical properties such as morphology, compressive modulus, water absorption capacity, and remaining weight after degradation. The compressive modulus increased from ~ 58 kPa for the PCL scaffold to ~287 - 1,861 kPa for PCL-PHB and PCL-PHBV dual-leached scaffolds. Mechanical properties of PCL-PHB and PCL-PHBV dual-leached scaffolds have been greatly improved by blending of PHB or PHBV. For the degradation observation, the remaining weight of PCL, PCL-PHB and PCL-PHBV

dual leached scaffolds were much decreasing compare to the absence of lipase condition. These results could be suggested that the activity of this enzyme lipase exhibited an effect to PCL, PCL-PHB, and PCL-PHBV dual leached scaffolds. Indirect cytotoxicity evaluation of these scaffolds with mouse fibroblastic cells (L929) and mouse calvaria-derived pre-osteoblastic cell (MC3T3-E1) indicated biocompatibility of these materials to both types of cell. The potential for use of these scaffolds as bone scaffolds was further assessed in vitro in terms of the attachment, the proliferation, the alkaline phosphatase (ALP) activity, and the mineralization of MC3T3-E1 that were seeded or cultured at different times. The obtained results showed that the both PCL-PHB and PCL-PHBV scaffolds exhibited better adhesion of cells than the TCPS and the PCL-PHB and PCL-PHBV dual-leached scaffolds exhibited better proliferation of cells than the corresponding TCPS and other scaffolds. Evidently, the cells that were cultured on all of the PCL-PHB and PCL-PHBV dual-leached scaffolds at 4 h appeared to be well-expanded and attach on scaffolds surface while that seeded on glass substrate was still in round shape. The cells that were cultured on PCL-10%PHB dual-leached scaffold showed the most expansion on the surface of scaffold. In mineralization assessment of MC3T3-E1 on days 14 and 21, the most intensity of staining product for calcium deposition were observed on PCL-PHB, and PCL-PHBV scaffolds compared to PCL scaffold and TCPS. Our results indicate that PCL-PHB and PCL-PHBV dual-leached scaffold possesses improvement in mechanical properties and degradation rate and its ability to support MC3T3-E1 cell attachment, proliferation, and mineralization for used as bone scaffolding material.

In this study, PCL-PHB/HA and PCL-PHBV/HA dual-leached scaffolds have been prepared by using solvent casting and salt particulate leaching with polymer leaching technique. Scanning electron microscopy (SEM) images confirmed that these scaffolds were characterized by highly interconnected networks, porosity, and a pore size of 367-389 μm. The mechanical properties, porosities, pore volumes, water absorption capacities, and weight remaining after degradation were also investigated. An indirect cytotoxicity evaluation with mouse fibroblastic cells (L929) and mouse calvaria-derived pre-osteoblastic cell (MC3T3-E1) revealed that the

scaffolds fabricated using our method released no substances at levels that were harmful to the cells. The potential for the use of these constructs in bone tissue engineering was evaluated in vitro using mouse calvaria-derived pre-osteoblastic cells (MC3T3-E1) that were seeded or cultured at different time. For the attachment and proliferation of the cells, all PCL-PHB/HA and PCL-PHBV/HA dual-leached scaffolds are able to support the proliferation of the cells at significantly higher levels to that on TCPS and PCL/HA, this results could be suggested that the PCL-PHB/HA and PCL-PHBV/HA dual-leached scaffolds provided better support for bone cell adhesion and proliferation. For the cells that were seeded on the surface of all scaffolds, the majority of cells appeared to be well-expanded and attach on scaffolds surface while that seeded on glass substrate was still in round shape on 4 h. At 1, 2, and 3 days after cells seeding, the majority of the cells seeded on the surfaces of all types of scaffolds expanded over the area of the scaffolds. In mineralization assessment of MC3T3-E1 on days 14 and 21, the most intensity of staining product for calcium deposition was observed on PCL-20%PHB/HA scaffold. Our results indicate that PCL-PHB/HA and PCL-PHBV/HA dual-leached scaffolds possess its ability to support MC3T3-E1 cell attachment, proliferation, and mineralization for used as bone scaffolding material.

Sodium chloride (NaCl) and polyethylene glycol (PEG) were used as water-soluble porogens for the formation of porous polycaprolactone (PCL) and its blends scaffolds. The main purpose was to prepare and evaluate in vitro efficacy of highly interconnected, three-dimensional, porous polymeric scaffolds, as obtained from the combined solvent casting and particulate-polymer leaching techniques. Evidently, the use of PEG as the secondary porogen not only improved the interconnectivity of the pore structures but also resulted in the scaffolds that exhibited much better support for the proliferation and differentiation of the cultured bone cells.The results indicate that all dual-leached scaffolds possess improvement and show their ability to support MC3T3-E1 cell attachment, proliferation, and mineralization for used as bone scaffolding materials.

7.2 Recommendations

Although the dual-leached porous scaffold showed good support for the attachment and proliferation of the cultured cells, but they are still needed to be improved. The improvement could be done by surface modification of the scaffold, by cell growth factor impregnation, or by biologically active molecules attachment to the scaffold.

In addition, the scope of the biological evaluation in this work focused only on the in vitro assessment. For further study to develop the scaffold for uses as the scaffolding material, an in vivo or animal study should be carried out.

Another recommended study may be concerned about the copolymers or blends of PCL, PLA, PGA, and other biodegradable polymers in order to obtain the scaffolds with optimal degradation rate for bone regeneration.