CHAPTER VIII

CONCLUSIONS AND RECOMMENDATIONS

In the present study, we developed a new biomaterial effective for bone regeneration consisting of electrospun (e-spun) fiber mats of poly(lactic acid) (PLA; M_n = 80,000 g·mol^{-1}) with collagen peptides bonded covalently. Surface modification techniques have been instrumental in the development of scaffolds that promote cell-surface interactions. Surface modification of PLA in the form of electrospun fibrous membranes (fiber diameters ~0.63 ± 0.1 μm; thickness ~136 ± 5 μm), was achieved by using the amino groups (NH_2) introduced on the scaffold surface via aminolysis with HMD as precursors. The reactive groups were subsequently used to graft extracellular matrix molecule, collagen type I, by using DSC as coupling agent. The existence of NH_2 groups was verified quantitatively by absorbance spectroscopy, ninhydrin analysis method, which revealed that the free amino group density was influenced by the HMD concentration and aminolyzing time. XPS analysis confirmed the presence of the biomolecule, collagen, on the surface of the polymer as indicated by the increase in the nitrogen/carbon ratio on the surface of the polymer, suggested that the modified PLA scaffolds became enriched with nitrogen atoms. In addition, water contact angle was measured to evaluate the wettability of the modified surfaces. Results showed that the hydrophilicity of the surface has improved obviously after aminolysis and collagen immobilization. The cytocompatibility of modified PLA scaffolds might be improved.

Therefore, the potential for use of the neat, the aminolyzed, the activated, and the collagen-immobilized PLA fibrous scaffolds as bone scaffolds was evaluated *in vitro* with mouse calvaria-derived, pre-osteoblastic cells (MC3T3-E1). Cell viability was estimated by the MTT assay and cell function were assessed by measuring alkaline phosphatase (ALP) secreted by MC3T3-E1. Indirect cytotoxicity evaluation revealed that both the neat and the modified PLA fibrous scaffolds released no substances at levels that were harmful to these cells. The number of cells attached on all types of the fibrous scaffolds at any given time point was lower

(~50%) compared with that on TCPS. While, MC3T3-E1 proliferation was improved remarkably on the modified surface, with the cells growing on the collagen-immobilized PLA fibrous scaffolds showing the greatest proliferation on day 3 after cell culture. SEM images showed that there are different in the morphology of the cells attached to the modified and unmodified scaffold surfaces. It was found that cells cultured on the unmodified substrate tend to become round on 4 h after cell seeding. While the cells which seeded on the modified scaffold were evidently expanded, with collagen-immobilized PLA surface did so to a greater extent. However, after 16 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 fibrous scaffolds reached a maximal value on day 5 after cell culture. The ALP activity of the cells grown on neat PLA fibrous scaffold was the lowest at any given time point. While among the various e-spun scaffolds investigated, collagen-immobilized PLA fibrous scaffolds showed the highest ALP activity, followed by the cells grown on the activated and the aminolyzed PLA, respectively. The obtained results showed that the collagen-immobilized PLA fibrous membrane is a good candidate to be used as a bone scaffold because it supported cell proliferation, and enhanced function of osteoblast-like cells.

The recommendation of the future work is based on an additional long term experiments in order to clarify the effect of scaffolds on the differentiation of osteoblasts (i.e., mineralization) should be carried out. Besides, the *in vivo* investigation of bone regeneration with implantation of these e-spun scaffolds should be done. Another recommended study may be concerned about the copolymers or blends of different chiral nature form of lactic acid which are poly-L-lactide (PLLA), poly-D-lactide (PDLA), and poly-DL-lactide (PDLLA) in order to obtain the scaffolds with optimal degradation rate for bone regeneration.