CHAPTER VIII
CONCLUSIONS AND RECOMMENDATIONS

In this research work, electrospun (e-spun) fiber mats of polycaprolactone (PCL; M_n = 80,000 g·mol^{-1}) with or without the presence of calcium carbonate ($CaCO_3$) or hydroxyapatite (HAp) nanoparticles (i.e. E-PCL, E-PCL/$CaCO_3$, and E-PCL/HAp) were successfully fabricated from the PCL solution (i.e., 12% w/v in 50:50 v/v dichloromethane/*N*,*N*-dimethylformamide). HAp was successfully synthesized by hydrolysis method, with the average particle size based on visual observation being about 230 nm. The average fiber diameters of the e-spun fibers were found to increase with the addition and increasing amount of the nanoparticles, which was caused by the increasing of the viscosity of the spinning dopes. The observed increase in the diameters of the e-spun fibers with the addition and increasing amount of the nanoparticulate fillers was responsible for the observed increase in the tensile strength of the obtained fiber mats. The concentration of the base PCL solution had a strong effect on the size of the e-spun fibers, in which the average diameter of the E-PCL/HAp composite fibers was found to increase with increasing concentration of the base PCL solutions. Increasing applied electrical potential also resulted in an increase in the diameters of the obtained E-PCL/HAp composite fibers. Indirect cytotoxicity evaluation of the E-PCL, E-PCL/$CaCO_3$, and E-PCL/HAp fibers based on human osteosarcoma cells (SaOS2) and mouse fibroblasts (L929) revealed that these e-spun mats posed no threats to the cells, which, in turn, implied their potential utilization as bone scaffolding materials. The potential use of these e-spun fiber mats as bone scaffolds was evaluated *in vitro* with human osteosarcoma cells (SaOS2), mouse calvaria-derived, pre-osteoblastic cells (MC3T3-E1), and human fetal osteoblasts (hFOB).

The E-PCL, E-PCL/$CaCO_3$, and E-PCL/HAp fibers were evaluated in terms of attachment, proliferation, and alkaline phosphatase (ALP) activity of the SaOS2 cells that were cultured directly on the scaffolds. The results were compared with those on corresponding solution-cast film scaffolds and tissue-culture polystyrene plate (TCPS). All of the e-spun fibrous scaffolds promoted much better adhesion and proliferation of cells than the corresponding film scaffolds and TCPS. Among

the various e-spun scaffolds investigated, the E-PCL/HAp (the one that was filled with 1.0% HAp) showed the highest ALP activity. Due to the observed highest ALP activity from SaOS2 cultured on the E-PCL/HAp scaffold, it is of our great interest on the E-PCL/HAp scaffold, which was further investigated with MC3T3-E1 cells.

The E-PCL and E-PCL/HAp were evaluated in terms of attachment, proliferation, differentiation, and mineralization of MC3T3-E1. The number of cells attached and proliferated on both types of the fibrous scaffolds at any given time point was lower (~50%) compared with that on TCPS. However, both of the fibrous scaffolds were able to support the proliferation of MC3T3-E1 at similar levels to TCPS, with the cells growing on E-PCL/HAp showing the greatest proliferation rate on day 3. Due to the much greater number of cells attached and proliferated on TCPS, ALP activity of MC3T3-E1 grown on TCPS reached a maximal value on day 3 after cell culture, while that of the cells grown on E-PCL/HAp did so day 5. On the contrary, the ALP activity of the cells grown on E-PCL was the lowest at any given time point, despite the relatively greater number of cells attached on its surface than that on the E-PCL/HAp counterpart. MC3T3-E1 cultured on the surface of E-PCL/HAp exhibited the greatest amount of osteocalcin (OC) gene on day 14 after cell culture and the greatest amount of OC protein on day 21 after cell culture, when compared with those cultured on the surfaces of E-PCL and TCPS. This translated to the greatest extent of mineralization for the cells grown on the surface of E-PCL/HAp on day 21, followed by that for the cells grown on E-PCL and TCPS, respectively.

The potential use of the E-PCL/HAp fiber mats as bone scaffolds w as further evaluated in terms of the proliferation, differentiation, and mineralization with human fetal osteoblasts, hFOB. Surfaces of TCPS and porous poly(DL-lactic-co-glycolic-acid) (PLGA) discs were used as control. The E-PCL and E-PCL/HAp supported growth, differentiation and mineralization of human fetal osteoblasts (hFOB). Compared to E-PCL and E-PCL/HAp, cell proliferation at day 7 was significantly higher on TCPS, and significantly lower on PLGA. Between E-PCL and E-PCL/HAp, cell proliferation was significantly higher on E-PCL/HAp. ALP activity at day 7 was significantly lower on TCPS compared to the all other materials (i.e. E-PCL, E-PCL/HAp, and PLGA) but there were no differences among the tested

materials. Calcium contents at 4 weeks from hFOB cultured on E-PCL, E-PCL/HAp, and PLGA were significantly higher than that on TCPS. However, there was no significant difference among E-PCL, E-PCL/HAp, and PLGA.

For the overall results, the E-PCL/HAp scaffold supported cell attachment, proliferation, and interestingly enhanced differentiation and mineralization. HAp is often used as a reinforcing bioactive agent to improve both the mechanical properties and osteoconductivity of PCL scaffolds (Kim, 2005; Chim, 2006). The enhanced differentiation and mineralization of osteoblast-like cells could be due to the presence of HAp, a synthetic calcium phosphate ceramic that mimics the natural apatite composition of bone and teeth, in which many research found that HAp help increase proliferation and mineralization of osteoblasts (Di Silvio, 2002; Kong, 2005). The obtained results showed that the E-PCL/HAp is a good candidate to be as a bone scaffold because it supported cell proliferation, and enhanced differentiation and mineralization of osteoblast-like cells.

The recommendation of the future work is based on *in vivo* investigation of bone regeneration with implantation of these e-spun scaffolds. From the preliminary results of *in vivo* study, the regeneration of rat cranium with the defect size of 8 mm was observed after 6 weeks of implantation of the E-PCL/HAp mats. The cross sections of bone specimens were analyzed histologically. The calcified bone matrix, orange stained surface, was observed on both upper and lower surfaces of scaffold with tendency of extension of bone formation into the scaffold (see Figure 8.1). Moreover, the biocompatibility of the E-PCL/HAp scaffolds was revealed by the appearance of the soft tissue portion, or extracellular matrix, which referred as the blue area of stained surface, at the close contact areas of the surface of scaffold (see Figure 8.2).

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.

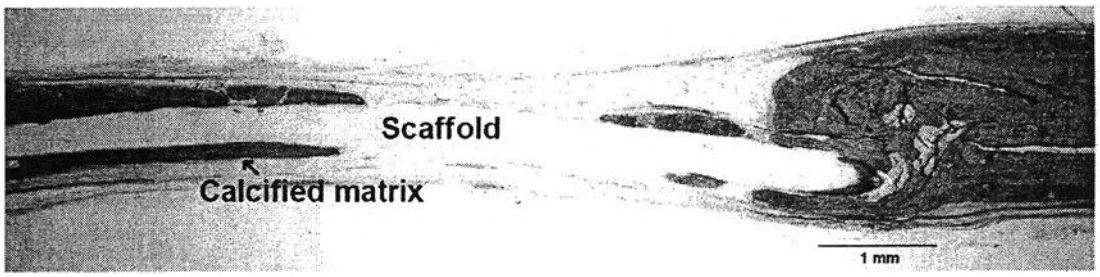

Figure 8.1 Stained cross-sectional surface of rat cranium after 6 weeks of implantation with the E-PCL/HAp scaffold. The defect size was 8 mm. A scale bar is 1 mm.

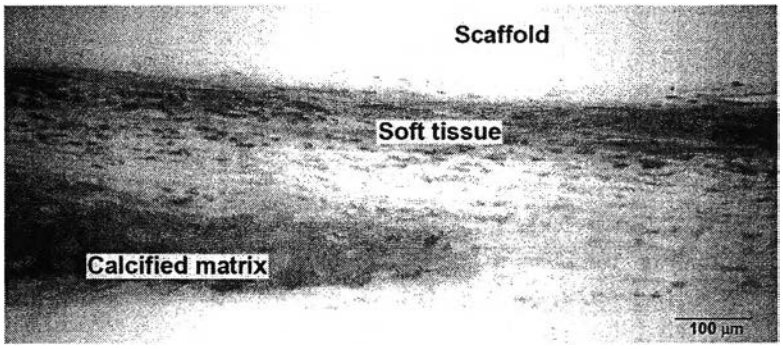

Figure 8.2 Stained cross-sectional surface of rat cranium after 6 weeks of implantation with the E-PCL/HAp scaffold. The defect size was 8 mm. A scale bar is 100 μm.