CHAPTER VII
CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

Electrospun nanofibrous membranes of chitosan or poly(N-acetyl-D-glucosamine-co-D-glucosamine) with the %DD of about 85% and the weight- and the number-average molecular weights of 610 and 110 $kg \cdot mol^{-1}$, respectively, were prepared from 7% w/v chitosan solution in 70:30 v/v TFA/DCM. Smooth fibers with the diameters of the individual fiber segments being 126 ± 20 nm were obtained. The membranes were evaluated for their potential for use as substrates for cell/tissue culture on four different cell lineages, i.e., Schwann cells, osteoblast-like cells, keratinocytes and fibroblasts, against the corresponding solvent-cast films. Both types of the chitosan substrates supported the attachment and the proliferation of keratinocytes very wells. Despite the poor attachment on the substrates, Schwann cells proliferated marginally well on these substrates. Finally, despite the good attachment of osteoblast-like cells, both osteoblast-like cells and fibroblasts were not able to proliferate on these substrates.

The effect of surface properties of various surface modified electrospun fibrous and solution-cast film PHB substrates were also evaluated *in vitro* towards murine neuroblastoma Neuro 2a cell line. The results suggested that the alteration of surface topography and chemistry has a significant impact on adhesion and proliferation of cells. The introduction of contact guidance such fiber alignment and biochemical mediation such immobilization of adhesive protein enhanced the attachment and proliferation of the cells. On laminin immobilized fibrous substrate, Neuro 2a aligned and elongated unidirectionally along the fiber axes. Some of cells wrapped around the individual fibers, while others anchored to multiple fibers. This result showed a greater preference toward fibrous surfaces over flat ones. However, the unmodified substrates of both film and fibrous substrates exhibited cytostatic property in which inhibited the cellular proliferation.

Immobilization of laminin on the surface of the electrospun PHB fibrous scaffolds was successfully accomplished by using the amino and carboxylic groups

introduced on the scaffold surface via surface-specific lysis reaction and further covalent immobilization of the biomolecules. The potential for use of the surface modified fibrous scaffolds for neural regeneration was evaluated *in vitro* towards murine neuroblastoma Neuro 2a cell line (ATCC, CCL-131). However, for potential uses in specific application, all of the fibrous scaffolds were further evaluated with rat brain-derived neural stem cells (NSCs). Both types of laminin coupled on the PHB fibrous scaffold supported the attachment and the proliferation of Neuro 2a very wells. Despite the good attachment and proliferation of Neuro 2a, NSCs were not able to proliferate on the neat PHB, H-PHB and LH-PHB fibrous scaffold.

Finally, we have successfully prepared the PHB-Fe_3O_4 composite nanofibers by combining electrospinning technology with *in situ* co-precipitation method. The Fe_3O_4 nanoparticles can be in-situ synthesized and well dispersed on the fibrous surface. The average particle sizes of the Fe_3O_4 nanoparticles were in the range of 67-82 nm. The particle size and particle size distribution of Fe_3O_4 nanoparticles were controllable by adjusting the concentration of aqueous iron ion solution. The obtained composite nanofibers have superparamagnetic properties with the saturation magnetization ranged from 0.13 to 0.60 emu·g^{-1} with very low remnant magnetization (0.025-0.125 emu·g^{-1}) and coercive fields (12-17 G) at room temperature. The potential for use of the obtained composite nanofibers as scaffolding materials for skin and nerve regeneration was further assessed with L929 and Neuro2a in terms of cytotoxicity after they were cultured in the extract media for different of immersion. The viability of L929 and Neuro2a cultured with the extraction media from all of composite nanofiber mats were equivalent to that of the cells cultured with fresh SFM. These results indicate that the PHB-Fe_3O_4 composite nanofibers can be served as ideal candidates for biomedical applications.

7.2 Recommendations

Due to the complicate interaction between materials and biological system, there is no precise measurement of effective scaffolding materials. Therefore, the understanding in the influence of surface properties on the cellular behavior is a

crucial role for developing the functional scaffolds. From the *in vitro* studies, the artificial scaffolds with different surface properties may provide the different of cellular responses. Thus, it is important that the obtained scaffolds should be thoroughly evaluated in vitro such in an animal.