CHAPTER VI
CONCLUSIONS

6.1 Conclusions

Hexanoyl chitosan (H-chitosan) was synthesized via a heterogeneous acylation reaction between chitosan and hexanoyl (caproyl) chloride. Films of H-chitosan were prepared and tested for their physical and biological properties. In addition, H-chitosan was successfully fabricated into nonwoven-fibrous mats composed of nano- and submicron-sized fibers through the electrospinning process. The mats were evaluated for their biocompatibility with living cells and for their potential use as biocompatible dressing or scaffolding materials. With all of the obtained results, it can be concluded that the electrospun fibers of H-chitosan are biocompatible with living cells and have potential to be developed as the material for dressing or scaffolding applications.

6.1.1 Synthesis and Characterization of Hexanoyl Chitosan

Hexanoyl chitosan (H-chitosan) was prepared by a heterogeneous acylation reaction of the chitosan with hexanoyl chloride based on the method described by Zong et al.(2000). IR spectrum, as well as ^{1}H-NMR spectrum, of the as-synthesized hexanoyl chitosan (H-chitosan) showed additional peaks from the spectrum of the original chitosan at various wave lengths. These results indicated successful substitution of hexanoyl groups into glycosamine units of chitosan. However, the hexanoyl groups did not fully substitute into all of the hydroxyl and amino groups on chitosan molecules as revealed by the IR spectra and the elemental analysis (EA) result. The number of hexanoyl groups per glycosamine unit was 3.0 out of 4.0 of the fully substituted H-chitosan. Thermal stability of H-chitosan and chitosan films was evaluated by TGA. Chitosan film was found to degrade at ca. 297 °C, while H-chitosan film exhibited two steps in the loss of its mass at ca. 242 and 299 °C, respectively, which are in general accordance with the earlier report (Peesan, Supaphol, & Rujiravanit, 2005). The first step in the loss of mass was a result of the loss of the hexanoyl side groups, while the second corresponded to the thermal degradation of the main chain. The incorporation of the hexanoyl side chains into the

structure of H-chitosan makes H-chitosan more hydrophobic and consequently renders H-chitosan its solubility in some common organic solvents, e.g. $CHCl_3$. The surface hydrophobicity of H-chitosan film was higher than chitosan film as revealed by static contact angle measurement that H-chitosan film possessed the value of 76° versus the value of 71° of chitosan film.

6.1.2 Electrospinning of H-chitosan

Electrospun hexanoyl chitosan (H-chitosan) fibers with ribbon-like morphology and average diameters ranging from 0.64 to 3.93 μm were successfully prepared from solutions of H-chitosan in chloroform. Three main factors that affected morphology and size of the electrospun fibers were viscosity, conductivity, and surface tension of the solutions.

Since increasing the solution concentration from 4% to 14% w/v, the solution viscosity went up 58 times as a result of an increase in polymer chain entanglement. With increasing H-chitosan concentration which, in turn, the solution viscosity, the average fiber diameter was found to increase, while the bead density was found to decrease. An increase in the mass throughput with increasing applied electrical potential was responsible for increasing the average diameter of the as-spun fibers. Finally, addition of an organic salt, pyridinium formate, helped increase the conductivity of the spinning solution, which resulted in a general increase in the average diameter and a general decrease in the bead density of the resulting H-chitosan fibers..

6.1.3 Biocompatibility of H-chitosan

The biocompatibility of H-chitosan was assessed by evaluating its cytotoxicity as well as the attachment and proliferation of the cells cultured on the film and electrospun mat of H-chitosan. The indirect cytotoxicity test with L929, mouse fibroblast-like, cells revealed that H-chitosan film and electrospun mats showed no toxicity and did not release substances harmful to the living cells. In addition, H-chitosan film could support the attachment and proliferation of L929 cells even better than chitosan film which is well known for its biocompatibility for years. As composed of a submicron-sized fibrous structure with interconnected pores mimicking the extracellular matrix (ECM) for tissue formation, the electrospun mat of H-chitosan was further assessed for its potential use as biocompatible dressing or

scaffolding, using human keratinocytes (HaCaT) and human foreskin fibroblasts (HFF). It was found that the H-chitosan fibrous scaffolds showed much greater viability of both the seeded and the proliferated cells than the film counterparts did and both the fibrous and the film scaffolds showed much better support for the attachment and the proliferation of HaCaT than those of HFF. Visual observation based on scanning electron microscopy revealed that both types of cells integrated well with surrounding fibers to form a three-dimensional cellular network and that the cells maintained their characteristic morphology during the cell culture. Since HaCaT and HFF are anchorage-dependent cells, the high surface area-to-volume ratio and the overall porous structure of the electrospun fiber mats are favorable parameters for promoting the attachment and the proliferation of the cells. As a result, cellular responses of both types of cells on the fibrous scaffolds were superior to those on the film counterparts.

7.2 Recommendations

Although the electrospun fibrous mats of H-chitosan 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 electrospun fibers, 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 wound dressing or scaffolding material, an in vivo or animal study should be carried out.