CHAPTER V

CONCLUSIONS AND SUGGESTIONS

Charged derivatives of chitosan, *N*-[(2-hydroxyl-3-trimethylammonium) propyl]chitosan chloride (HTACC), *N*-succinyl chitosan (SCC), and *N*-sulfofurfuryl chitosan (SFC) were prepared by ring opening of GTMAC, ring opening of SA by amino groups of chitosan, and reductive alkylation using FFSA as reagent, respectively. Percentage of the degree of substitution (%DS) of all derivatives can be varied as a function of reagent equivalent in comparison with the amino groups of chitosan. ^{1}H NMR and FTIR analyses were used to verify the chemical structures of the charged derivatives.

QCM analysis suggested that multilayer films of these 3 pairs of polyelectrolytes: HTACC-PAA, PAH-SCC and PAH-SFC have been formed by alternate layer-by-layer adsorption. The assembly process was driven by electrostatic attraction and hydrogen bonding. It was also found that the thickness of the adsorbed HTACC and SFC and their multilayer films depended upon %DS. The greater the %DS, the more chance the polymer chains adopted more extended conformation, and the thinner the adsorbed layer. The characteristic functional groups of polyelectrolytes in the multilayer films and the stratification of the multilayer films deposited on the plasma-treated poly(ethylene terephthalate) (treated PET) substrates were verified by ATR-FTIR analysis and water contact angle measurements, respectively. As characterized by AFM, the multilayer films can homogeneously cover the substrate and have the ability to smooth-out a rough surface of the treated PET substrates. However, the substrates became increasingly rougher as the number of the deposition was greater than 9 in the case of PAH-SFC system.

Biological responses of the assembled films as assessed by *in vitro* cell adhesion and proliferation of fibroblasts showed that the degree of cytocompatibility of the multilayer films depends strongly on the polyelectrolyte used for the assembly process and the last layer deposited. The multilayer films having charged derivatives

of chitosan (HTACC, SCC, SFC) as a top layer were less favorable substrates for cell adhesion and proliferation than those having oppositely charged synthetic polyelectrolytes (PAH, PAA). It is believed that the opposite charges of HTACC can bind strongly with the cells and causes adverse effect on adhesion and proliferation of the cells. SCC and SFC, on the other hand, carried some negative charges, which is the same charge as the cell membrane. The charge-charge repulsion may be used to explain why it is unfavorable for cell attachment. Although none of the charged derivatives of chitosan promoted cell attachment and proliferation, it does not mean that they are useless. Materials that prevent cell adhesion may be useful for some biomedical applications.

Future work will cover the viscoelastic properties of polyelectrolyte multilayers using quartz crystal microbalance with dissipation (QCM-D). The information will be very useful for understanding the adsorption behavior of the charged derivatives of chitosan, i.e. how they would affect their biological responses. Anticoagulant and antibacterial activities of the multilayer films assembled from charged derivatives of chitosan are also interesting issues for future investigation. These studies should help broaden applications of chitosan, especially in the field of biomaterials and biomedical applications.