CHAPTER V

CONCLUSION AND SUGGESTION

Immobilization of RGD-containing peptides on the surface of poly(DTE-*co*-20%DT carbonate) was successfully accomplished through a two-step reaction. The first step involved an activation of carboxyl groups by *N*-hydroxysuccinimide (NHS) in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI). The activation of the poly(DTE-*co*-20%DT carbonate) surface with NHS was characterized by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. An approximated bulk yield of 20% was obtained using the EDCI/NHS concentration of 0.1 M and a reaction time of 24 h in ethanol.

Reaction with L-3,5-dibromotyrosine, a model peptide, has demonstrated that the activated carboxyl group in the form of *N*-succinimide ester was quite stable, yet reactive enough to interact with amino groups of the model peptide giving reasonably high % substitution (~92%) The success of peptide immobilization in the second step was determined by X-ray photoelectron spectroscopy (XPS) and ninhydrin method. Using 1,6-hexamethylenediamine as a standard, the graft density of ~ 8.07×10^{-8}, 6.51×10^{-8}, and 5.13×10^{-8} mol/cm^2 was estimated for immobilization with RGD, RGDS, and GRGDS, respectively. According to XPS analysis of RGD-immobilized poly(DTE-*co*-20%DT carbonate) surface after labeling with heptafluorobutyryl chloride, 30 and 75 % substitution were calculated for immobilization with RGDS and GRGDS, respectively.

Results from *in vitro* cell studies suggested that the alteration of surface functionality has a significant impact on fibroblast (B95) adhesion and proliferation. Among all studied RGD-containing peptides, GRGDS can best enhance cytocompatibility of the polymer surface. Taking 100% of TCPS as a positive control, cell adhesion and proliferation ratios were elevated from 92.3 and 89.6% of the virgin polymer to 117.0 and 122.6% respectively, after GRGDS immobilization. The extra

glycine spacer presumably introduces the flexibility to the peptide and thus allows the RGD part to effectively mediate its specific response to the cells. This success would expand the applicability of this polymer in biomedical applications especially those requiring specific cellular response.

For future studies, attachment of other peptide sequences to improve specific cellular responses of poly(DTE-*co*-20%DT carbonate) surfaces are desirable. RGD-containing cyclic peptides are considered as potential candidates for improving cellular responses since their suitable conformation allows the RGD sequence to efficiently mediate its specific response to cells.