CHAPTER V
CONCLUSIONS

In this research, well-defined random and block copolymers consisting of activated esters (*N*-acryloxysuccinimide, NAS and pentafluorophenyl acrylate, PFPA) and *N*-isopropylacrylmide (NIPAAm) were first synthesized by reversible addition-fragmentation transfer (RAFT) polymerization. ^{1}H NMR and FTIR measurements proved a successful formation of the copolymers. In addition, kinetic analysis and gel permeation chromatography (GPC) measurements confirmed that polymerization was well controlled.

Light responsive moieties of o-nitrobenzyl (ONB) were introduced to the copolymers via post functionalization of the activated esters part in the copolymers. The ONB groups can be released upon UV irradiation at 365 nm which subsequently induced an *in-situ* cross-linking by a spontaneous reaction with the remaining activated esters and yielded stable network. The successful conversion of the activated esters in the copolymer to the corresponding ONB group was confirmed by ^{1}H NMR, FTIR, and GPC measurements.

The core cross-linked amphiphilic polymeric micelles of PPFPA-*b*-PNIPAAm were prepared via post polymerization modification of PFPA moieties with the ONB groups. The size of the uncross-linked and cross-linked micelles block copolymers having M_n of 30 kDa can be controlled by changing the external temperature above or below their LCSTs. Dynamic laser scattering (DLS) proved a successful formation of the micelles as well as transmission electron microscopy (TEM) showed that the micelles were in well-defined spherical shapes.

Furthermore, the ONB-containing random copolymer of P(NIPAAm-*r*-PFPA) and P(NIPAAm-*r*-NAS) could be electrospun to form stable cross-linked fibrous structure after UV irradiation. The *in vitro* cytotoxicity of GRGDS peptide-immobilized uncross-linked and cross-linked (GRGDS-UC and GRGDS-C) of P(NIPAAm-*r*-PFPA) fibers was performed against mice L929 fibroblast cell line using MTT assay. The cell adhesion was determined at 6 h and cell proliferation was evaluated at 1 day, 3 days and 5

days of cell culture. After 6 h of cell culture, the percent cell adhesion ratio became higher after the fibers were immobilized with GRGDS peptide for both of uncross-linked and cross-linked fibers indicating that the GRGDS peptide can support cell adhesion.

Furthermore, cell proliferation increases with an increase in cell culture time from 1 day to 5 days. The percent of live cells for GRGDS-C fibers was higher than those of GRGDS-UC fibers. These results suggested that not only peptide but also hydrophilicity of the surfaces are important parameters that dictate the cell growth. Moreover, spread cells were more slightly detached from GRGDS-C surfaces than GRGDS-UC surfaces probably due to the GRGDS-C surfaces are more hydrophilic than that of the GRGDS-UC surfaces. The cellular responses suggested the possibility of using the developed GRGDS-C fibers as scaffolds for cell surface adhesion control in tissue engineering.

For future studies of micelles, incorporating of other active functional molecules such as dyes, drugs, or proteins into the core of cross-linked micelles via post functionalization modification may be useful to achieve new amphiphilic polymeric micelles for advanced applications. In addition, attachment of other peptide sequences or carbohydrate molecules onto the electrospun surfaces is also desirable to improve specific cellular responses for tissue engineering application.