# SYNTHESIS OF BLOCK COPOLYMER CONTAINING POLYPEPTIDE AND ITS BIOMEDICAL APPLICATION

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## 1. Introduction

Studies of synthetic polypeptides have been extensively investigated as the model of proteins because of the rather complicated structure of proteins. And they have been applied as implanted materials(1), materials for cell separation(2) and drug delivery matrix(3) due to their good biocompatibility or biodegradability. Block copolymers comprising two kinds of incompatible polymer chains usually undergo phase separation. Gallot et al.(4) first carried out a study on the synthesis and microheterophase structure determination of AB-type diblock copolymers consisting of a polypeptide block and a vinyl polymer. Nakajima et al.(5) synthesized ABA-type triblock copolymers consisting of polypeptide blocks as the A component and polybutadiene as the B component, which gave a microheterophase structure. The microheterophase structure and thrombogenic properties of ABA block copolymers containing polypeptide blocks were reported by Barenberg et el.(6). A study of ABA block copolymers consisting of incompatible A and B polymer chains is of interest with special reference to their biocompatibility or antithrombogenicity, because microphase separation of block copolymers consisting of hydrophilic and hydrophobic chains is reported to play a role in antithrombogenicity(7).

In this research, synthesis and structural study of an ABA block copolymer consisting of synthetic polypeptide as the A block and polyether as the B block will be reported. Also, antithrombogenic properties, drug delivery system of the block copolymers and cell attachment onto the copolymer will be reported for biomedical applications.

## 2.Experimental

### 2.1 Synthesis of poly(γ-alkyl L-glutamate)(PALG)/polyether/PALG block copolymers

It was prepared by polymerization of γ-alkyl L-glutamate N-carboxyanhydride(alkyl: methyl, ethyl and benzyl) initiated by the amine-terminated polyether[polyether: poly(ethylene oxide)(PEO) and poly(propylene oxide)(PPO)] in methylene dichloride(8,9). Poly(ε-benzyloxycarbonyl L-lysine)(PCLL)/PEO/PCLL was prepared by the similar method of PALG/PEO/PALG block copolymer(10).

2.2 Measurement of $^1$NMR spectroscopy

$^1$H NMR spectra of the copolymers were measured in a mixed solvent of $CDCl_3$ and trifluoroacetic acid to estimate the copolymer composition and the molecular weights of peptide blocks, using a JEOL FX 90 Q NMR spectrometer.

2.3 Observation of transmission electron microscopy(TEM)

A thin film was prepared at room temperature by casting the copolymer solution in $CHCl_3$ on a carbon film coated on a copper grid for TEM observation. After being stained with ruthenium tetroxide in a vapor phase, the specimen was subjected to TEM observation in a Hitachi HS-7 electron microscope.

2.4 In vitro evaluation of the antithrombogenicity on polymer surface

In vitro test was performed by the infusion syringe pump.

2.5 In vivo evaluation of the antithrombogenicity on polymer surfaces

The polymer-coated cathether was implanted in mongrel dogs for 1 hr and 48 hrs.

2.6 In vitro drug release studies

The release experiments were carried out in one ml phosphate buffered saline(PBS)(pH=7.4) in a shaking waterbath at 37°C. One ml aliquot was taken and replaced with fresh PBS at specific time points. The concentrations of the samples were determined by UV spectrometer.

2.7 Transferring of monolayers onto the substrate

Monolayers on the water surface were transferred onto a quartz plate by the horizontal lifting method, at surface pressure 3 dyn/cm. Monolayers were transferred only when the quartz plate was lowered in solution,i.e., the X-type deposition.

2.8 Cell adhesion

Copolymers prepared by the Langmuir-Blodgett(LB) film and solvent cast onto glass plates were immersed in separate platelet and hepatocyte cell suspensions and placed in an incubator with 5 wt% $CO_2$ at 37°C for 1h(11,12).