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Regulation of Nano-surface Properties by Finely Synthesized Fluorinated Phosphorylcholine Polymers

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A series of random and block copolymers composed of hydrophilic and hydrophobic monomer units have been synthesized by free and living radical polymerization methods, respectively. As the hydrophilic monomer unit, 2-methacryloyloxyethyl phosphorylcholine (MPC) was selected because the MPC polymers are well-known for their excellent bio- and blood compatibilities. The semi-fluorinated monomer, 2,2,2-trifluoroethyl methacrylate (TFEMA), was used as the hydrophobic monomer unit. Several analyses of the copolymer surface showed that the TFEMA unit was enriched at the outermost surface in the dry state on the random copolymer surface. Also, the microdomain structure was formed on the block copolymer surface. The reorientation of the MPC unit occurred dynamically in the wet state because of the strong hydrophilicity of the MPC units. Even in the case of the block copolymer with a low-MPC-unit composition, the surface was covered with the MPC units. As a result, the amount of the adsorbed bovine plasma fibrinogen on the block copolymer surface was reduced dramatically.

Key words: 2-methacryloyloxyethyl phosphorylcholine, fluorinated polymer, biocompatibility, protein adsorption, micrdomain structure

1. INTRODUCTION

Several surface modifications to conventional polymeric materials have been made using newly designed polymers because the functional groups at the surface play an important role in the interactions between the materials surface and biological molecules or cells [1,2]. However, on these studies, the biological phenomena in the biomolecules' scale, such as the amount or the structural change of the adsorbed biomolecules can be only discussed because the materials surface was modified in the micron order. For the future, it is necessary to control the interactions with the smaller portion, such as the receptor of the biomembrane. To realize this concept, the polymeric materials should be designed in the monomer unit order. The objective of this study is to control the surface structures and properties in the monomer unit order by the fluorinated phosphorylcholine polymers finely designed their sequence of the monomer units. As the fine design of the monomer unit sequence, the intramolecular gradient copolymer composed of the hydrophilic and hydrophobic monomer units is synthesized as well as the random and block copolymers.

As the hydrophilic monomer, 2-methacryloyloxyethyl phosphorylcholine (MPC) was selected. The MPC was prepared on a bioinspired concept. That is, if a biomembrane-like structure could be prepared on the surface, the surface would become biocompatible. We already found that the MPC polymers have an excellent bio- and blood compatibilities [3,4]. The semi-fluorinated monomer, 2,2,2-trifluoroethyl methacrylate (TFEMA), was used as the hydrophobic monomer. The fluorinated polymers have several unique characteristics, such as water- and oil repellence, low surface tension, the high affinity to oxygen molecules and chemical or thermal stability [5]. However, it is also known that the solubility of fluorinated polymers in several solvents is often low and their biocompatibility is not very high. Thus, the semi-fluorinated polymer with the amphiphilic and biocompatible MPC unit could improve the solubility and biocompatibility of the fluorinated polymers. The unique functionalities could also be assigned to the surface. Furthermore, the biocompatible surface could be constructed by the hydrophilic-hydrophobic microdomain structures with extremely large differences in the surface tensions between the domains.

Recent progress in the controlled living radical polymerization makes it possible to obtain polymers with narrow molecular weight distribution, active terminal groups and well-defined structures. Therefore, the living radical polymerization could be an indispensable way to synthesize well-defined polymers for controlling the structure of the biomaterial surface.

In this study, we synthesized the random and block copolymers composed of MPC and TFEMA

units with controlling their sequence, and their surface properties as the biomaterials were investigated.

2. MATERIALS AND METHOD

2.1. Materials

MPC was synthesized by a previously reported method [6]. TFEMA was obtained from Tosoh/F-tech Co., Tokyo, Japan, and was used without further purification. Other reagents and solvents were commercially available in extra-pure grade and were used without further purification.

2.2. Synthesis of PTFEMA-BDC macroiniferters

Benzyl N, N-diethyldithiocarbamate (BDC) as a photoiniferter was prepared according to the method previously reported by Otsu *et al* [7]. The TFEMA homopolymers introduced the iniferter (poly(TFEMA)(PTFEMA-BDC)) were synthesized by living radical polymerization. The BDC was placed in a flask and the TFEMA was added. The mixture was then diluted with 1,4-dioxane to adjust the concentration of the TFEMA to 0.50 mol/L. The concentrations of the BDC were adjusted to 17, 5.0 and 2.5 mmol/L. Argon was bubbled into the solution to eliminate oxygen.

The polymerization was carried out at room temperature under irradiation by UV light (UVL-400HA, Riko, Chiba, Japan) (λ = 360 ± 50 nm). After polymerization for 12 h, the content was poured into a large amount of hexane to precipitate the polymer. The precipitate was filtered off and dried in vacuo. The chemical structure of PTFEMA-BDC was confirmed by ^{1}H-NMR and FT-IR. The molecular weight of PTFEMA-BDC was determined by both ^{1}H-NMR and gel permeation chromatography (GPC). Tetrahydrofuran (THF) was used as an eluent for the GPC measurement at a flow rate of 1.0 mL/min. Polystyrene standard samples were used to determine the molecular weight.

2.3. Synthesis of block copolymers

A procedure similar to the synthesis of the macroiniferter was used for block polymerization with PTFEMA100-BDC as the macroiniferter. The PTFEMA100-BDC was synthesized when the concentration of the BDC was 5.0 mmol/L. The methanol/1,4-dioxane mixture (7/3 by volume) was used as a solvent, and the concentration of MPC was adjusted to 0.2 mol/L. The polymerization was carried out with various concentrations of PTFEMA100-BDC. The polymers formed were purified by reprecipitation, and the precipitated polymers were then dried in vacuo. The chemical structure of poly(MPC-*block*-TFEMA) (PMFb, Fig. 1) was confirmed by ^{1}H-NMR and FT-IR. The compositions of each component in the PMFb were determined from the phosphorous analysis.

2.4. Synthesis of random copolymers

The poly(MPC-*random*-TFEMA)s (PMTF) were

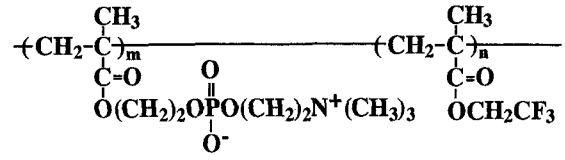

Fig. 1. Chemical structure of poly(MPC-*co*-TFEMA).

synthesized by free radical polymerization. The MPC and TFEMA were placed in a glass tube and 2,2'-azobisisobutyronitorile (AIBN) was added. The mixture was then diluted with ethanol to adjust the total volume to 30 mL, and the concentrations of the total monomer and AIBN were 0.50 mol/L and 5 mmol/L, respectively. Argon was bubbled into the solution to eliminate oxygen and then the glass tube was sealed.

The polymerization was carried out at 60 °C for 24 h. After the precipitation using a diethyl ether/chloroform mixture (8/2 by volume), the precipitate was filtered off and dried in vacuo. Poly(MPC)(PMPC) and poly(TFEMA)(PTFEMA) were also synthesized using ethanol and 1,4-dioxane as the polymerization solvents, while chloroform and hexane were used as the solvents for the precipitation, respectively. The chemical structures of PMTF, PMPC and PTFEMA were confirmed by ^{1}H-NMR and FT-IR. The mole fraction of each component in the PMTF was determined from the ^{1}H-NMR.

2.5. Polymer coating on substrates

Prior to the coating, 40 x 10 x 1.1 mm^3 glass plates were cleaned with hexane, acetone and methanol or ethanol and 40 x 10 x 0.95 mm^3 quartz plates with fuming nitric acid, water and acetone. These substrates were immersed in the polymer solutions (PMFb, 1.0 wt% in methanol; PMTF and PMPC, 0.5 wt% in ethanol; PTFEMA100-BDC and PTFEMA, 2.0 wt% in chloroform) twice (the interval was 30 minutes), dried under a solvent atmosphere overnight, and then vacuum-dried overnight. These samples were used for the analysis in the dry state. For the analysis in the wet state, every sample was stored in distilled water or phosphate-buffered solution (PBS, pH 7.4) for 1 day to equilibrate the surface, and then the sample was freeze-dried when it was needed.

2.6. X-ray photoelectron spectroscopic measurement

An X-ray photoelectron spectroscope (XPS, AXIS-HSi, Shimadzu/KRATOS, Kyoto, Japan) was used to determine the monomer unit composition at the polymer surface. The takeoff angle of photoelectrons was 15 degrees. The measurement was performed in both the dry state and the wet state after freeze-drying.

2.7. Transmission electron microscopic observation

On the dry state, the samples specially prepared were exposed to tetroxide (OsO_4) vapor to selectively stain the PMPC domain, and the

microdomain structure was observed by a transmission electron microscope (TEM, H-600, Hitachi, Tokyo, Japan).

2.8. Analysis of surface tension

The surface tension values are the sum of polar and non-polar components calculated from the static contact angles of water and diiodomethane on the sample surface [8]. The surface tensions of these liquids are summarized in Table I. The sessile drop technique (CA-W, Kyowa Interface Science Co. Ltd., Saitama, Japan) was used to measure the static contact angle (SCA) of liquids with known surface tension.

Table I. The surface tension of liquids and their polar and non-polar components (dyne/cm).

Liquid	γ_L^p	γ_L^d	γ_L
Water	43.7	29.1	72.8
Diiodomethane	4.0	46.8	50.8

2.9. ζ-potential measurement

The glass plates and the polymer surfaces were subjected to ζ-potential measurement using an ELS-800 electrophoretic light-scattering spectrophotometer (Otsuka Electronics, Osaka, Japan) equipped with a plate sample cell. The measurement was carried at 25 °C in water containing 10 mmol/L sodium chloride.

2.10. Evaluation of protein adsorption

The micro BCA method was used to determine the amount of adsorbed proteins on the sample surface [9]. The protein used in this study was bovine plasma fibrinogen (Fib). The concentrations of the Fib in PBS were 0.03 g/dL. This protein solution was placed in contact with the surface of samples at 37 °C for 2 h. To rinse these samples, they were immersed in PBS repeatedly 50 times. To detach the adsorbed proteins on the surface completely, the samples were immersed in a 1 wt% PBS solution of sodium dodecylsulfate (SDS) and applied sonication for 20 minutes using an ultrasonic water bath. The protein analysis kit (micro BCA (bicinchoninic acid) protein assay reagent kit, #23235, Pierce, Rockford, IL, USA) was used to determine the concentration of the proteins in the SDS solution, and the amount of the adsorbed proteins on the surface was calculated.

3. RESULTS AND DISCUSSION

3.1. Characterization of block and random copolymers

The PTFEMA-BDC was synthesized by living radical polymerization when the concentration of the BDC was varied. It was revealed that the molecular weight of the PTFEMA-BDC could be controlled by the concentration of the BDC.

The block copolymers were synthesized with the PTFEMA100-BDC as the macroiniferter. Table II shows the synthetic results for PMFb. The MPC unit composition of the PMFb reflected that of the feed. All the PMFbs were insoluble in water.

Table II. Synthetic results of poly(MPC-*block*-TFEMA).

Abb.	[M]/[S]	MPC unit mole fraction		Yield (%)
		In feed	In copolymer [a)]	
PMFb100-6	6	0.11	0.25	20
PMFb100-20	20	0.30	0.38	41
PMFb100-50	50	0.53	0.46	41
PMFb100-200	200	0.81	0.64	44

[M] = 0.2 mol/L
Polymerization time : 9h
a) Determined by phosphorus analysis

Random copolymers composed of MPC and TFEMA (PMTF) could be obtained by conventional radical polymerization. Table III shows the synthetic results for PMTF. The MPC unit composition of the PMTF was almost the same as that in the feed. The PMTFs with a high-MPC-unit composition were soluble in water.

Table III. Synthetic results of poly(MPC-*random*-TFEMA).

Abb.	MPC unit mole fraction		Molecular weight[b)]		Yield (%)
	In feed	In copolymer[a)]	Mn (x 10^{-3})	Mw/Mn	
PTFEMA	0	0	33[c)]	1.9[c)]	55
PMTF20	0.20	0.23	6.0	2.2	66
PMTF40	0.40	0.43	9.0	3.1	73
PMTF60	0.60	0.64	12	4.4	64
PMTF80	0.80	0.76	12	4.3	98
PMPC	1.0	1.0	5.0	3.3	89

[M] = 0.5 mol/L
[AIBN] = 5 mmol/L
Polymerization time = 24 h
a) Determined by ^{1}H-NMR measurement
b) PEG standard, MeOH / H_2O = 7 / 3 , [LiBr] = 10 mM
c) PSt standard, THF

3.2. Chemical analysis of the polymer surface

The samples prepared in this study were all transparent. The composition of each monomer unit on the outer surface of PMFb and PMTF was calculated by XPS both on the dry and wet state (XPS charts are not shown). The TFEMA unit was concentrated at the surface of PMFb100-200, PMTF40 and PMTF80, that is, the TFEMA unit composition at the surface was higher than that in the bulk on the dry state. When surfaces with the same monomer unit composition were compared, the MPC unit composition on the wet surface was larger than that on the dry surface regardless of the copolymer. This showed that the reorientation of the MPC unit occurred on the sample surface when they were soaked in water.

Fig. 2 shows the TEM image on the PMFb100-200 surface. The bright and dark regions show the PTFEMA and PMPC domain, respectively. The formation of the microdomain structure (its size was about 300 nm) was confirmed on the block

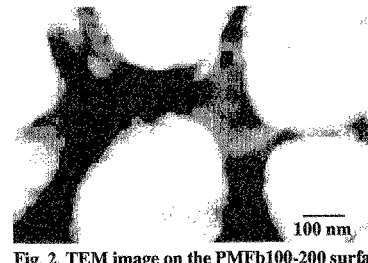

Fig. 2. TEM image on the PMFb100-200 surface. (bright region is the PTFEMA domain; dark region is the PMPC domain).

copolymer, while the microdomain structure could not be observed on the PMTF surface.

In the case of the PMFb, the polar component of the surface tension began to increase with a low-MPC-unit composition, while it began to increase with a high-MPC-unit composition in the case of the PMTF. This result confirmed that the microdomain structure was formed on the block copolymers.

To evaluate the surface property in the wet state, the ζ-potential measurement was carried out using PMFb and PMTF with a lower MPC unit composition. It is known that the ζ-potential of the surface covered with the MPC unit is about zero because the ionic group in the MPC unit forms an inner salt and the electrostatic effects are diminished. The ζ-potentials of the glass plate and the PTFEMA surfaces were both strongly negative. As the MPC unit composition of the PMTF increased, the ζ-potential of the surfaces became gradually close to zero. However, the ζ-potential of the PMFb surfaces became zero even on the surface with a low-MPC-unit composition. This result showed that all PMFb surfaces were covered with the MPC unit.

3.3. Protein adsorption on the surface

The amount of adsorbed Fib on the surfaces was determined by a micro BCA method after detachment of the adsorbed Fib. It was reported that the theoretical amounts of Fib adsorbed on the surface in a monolayer state are 0.27 mg/cm^2 [10]. As shown in Fig. 3, the amount of the adsorbed Fib on the surfaces was significantly reduced in a monolayer state by the introduction of the MPC unit compared with that on a PTFEMA, quartz or glass surface. The amount of the adsorbed Fib was suppressed on the PMFb surface with a low-MPC-unit composition, which was different from that on the PMTF surface with almost the same MPC unit composition. This result was consistent with the result of ζ-potential measurement.

4. CONCLUSIONS

The block copolymers, poly(MPC-*block*-TFEMA) (PMFb) composed of hydrophilic phospholipid and hydrophobic fluorinated segments, could be synthesized by living radical polymerization. According to the surface observation and analysis in the dry state, the microdomain structure was confirmed on the PMFb surface. In the wet state, the reorientation of the MPC unit occurred and even in the case of the PMFb surface with a low-MPC-unit composition, the surface was covered with MPC units. Therefore, the amount of the adsorbed Fib was lowered significantly on the PMFb compared with that on the corresponding random copolymer with the same MPC unit composition.

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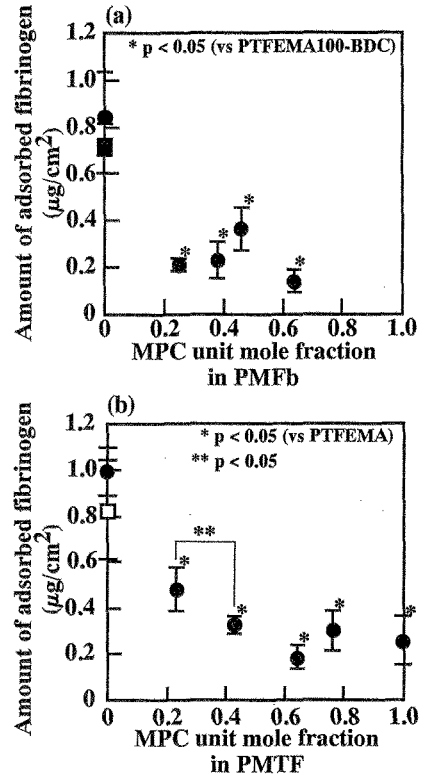

Fig. 3. Amount of adsorbed fibrinogen. (■ glass plate; □ quartz plate: (a) PMFb; (b) PMTF).

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