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Preparation of Biointerface on Nanoparticles Surface by Atom Transfer Radical Polymerization

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Biointerface composed of bimolecules with pH response, bioaffinity and protein immobilization functionalities using synthesized surface initiator coated on magnetic nanoparticles surface was successfully prepared by atom transfer radical polymerization (ATRP) in methanol at ambient temperature. The pH response polymer brush with 2-(diethylamino)ethyl methacrylate (DEA)-grafted magnetic nanoparticles can be dispersed in below pKa 7.3 aqueous solution due to protonation overcome magnetic interaction. On the other hand, polymer and block copolymer with bioaffinity and proteine immobilization functionalities based on 2-methacryloyloxyethyl phosphorylcholine (MPC)-grafted nanoparticles can be dispersed in neutral aqueous solution in spite of having a neutral charge. It is considered that steric repulsion of surface polymer brush has influenced the dispersibility. In the case of block copolymer composed of bioaffinity and protein immobilization functionalities, when the rate of immobilization of bovine serum albumin (BSA) was estimated, it turned out that the original protein immobilization part of surface polymer was exchanged for BSA. It is concluded that biointerface with various functionalities can be prepared by ATRP method to be useful for such as bioaffinity beads construction.

Key words: ATRP, biointerface, 2-methacryloyloxyethyl phosphorylcholine, magnetic nanoparticles

1. INTRODUCTION

Nanoparticles are widely applied as diagnostic reagents, drug-delivery carriers or bioaffinity beads [1, 2]. When we look ahead to the future, probably, it is needed to synthesize precisely designed beads surface containing such as thermal, pH, and light responsibility, high bioaffinity and capability to capture of small amount protein or specific protein among various protein.

On the other hand, atom transfer radical polymerization (ATRP) was widely used for polymer synthesis as a living free radical polymerization [3]. ATRP has advantages such as control of molecular weight by changing ratio of initiator to monomer concentration or reaction time, narrow polydispersivity, easy synthesis of block or triblock copolymer, preparation of high density polymer brush and so on under mild condition. In particular, as a "grafting from" method, ATRP has been used for direct polymerization on surface initiator coated solid surface such as silicon wafer [4]. Therefore, even if the nanoparticles cores are polymer, metal and ceramics, this method can be adaptable to any type of cores if surface initiator is fixable.

In this paper, preparation of biointerface on magnetic nanoparticles surface using by ATRP was reported. Here, two biointerfaces was prepared. One is pH response surface. Another is the surface which can immobilize protein. The magnetic particulate was chosen because of the wide adaptation range using magnetic interaction. Magnetic particles have been widely used in the study of in vivo biomedical applications, including magnetic resonance imaging [5] and markers of antigen-antibodies [6]. Fig.1 shows scheme on preparation of biointerface composed of biomolecules with pH response, bioaffinity and protein immobilization functionalities from magnetic nanoparticle surfaces using ATRP.

Previously, we reported the synthesis of the poly(L-lactic acid) particle coated with the random polymer which consists of randomly located bioaffinity unit (MPC), hydrophobic unit, and protein immobilization unit [7]. S. P. Armes et al., also reported preparation of magnetic nanoparticles coated with MPC block polymer that consists of bioaffinity and hydrophilic unit as the adsorbing block synthesized by ATRP [8]. Compared to the preparations, this method has advantage that can construct high-density functional polymer brush of purposive arrangement on the surface initiator fixed by chemical bond and make the protein immobilization part locate to be most outer surface that is most effective position for high capture ratio of protein due to use effectively the high specific surface area of nanoparticles.

2. EXPERIMENTAL

2.1 Material

2-(Diethylamino)ethyl methacrylate (DEA) monomer was purchased from Aldrich and purified by distillation under reduced pressure over calcium hydride. DEA was used as pH response monomer because DEA has around pKa 7.3 [9] that are near by body's internal environment. 2-methacryloyloxyethyl phosphorylcholine (MPC) was used as a bioaffinity monomer. MPC was synthesized using a method reported previously [10]. *p*-Nitrophenyloxycarbonyl polyethylene glycol methacrylate (MEONP) monomer was synthesized according to reference [11] and used as a protein

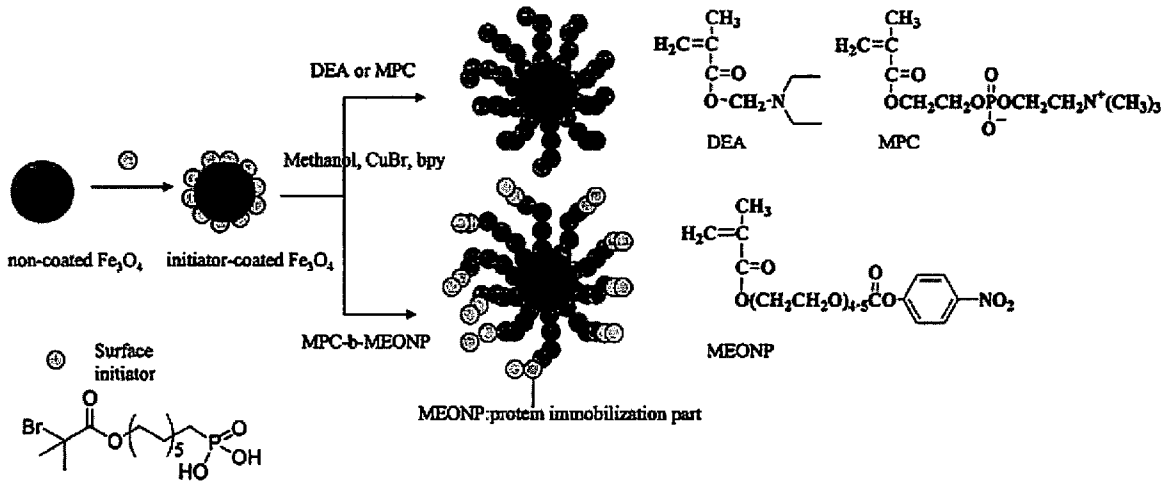

Fig.1 scheme on preparation of biointerface composed of biomolecules with pH response, bioaffinity and protein immobilization functionalities from magnetic nanoparticle surfaces using ATRP and structure of surface initiator, DEA monomer, MPC monomer and MEONP monomer.

immobilization part. The MEONP unit has an active ester group in the side chain, which reacts with proteins by the condensation with an amino group of the proteins [12]. Cu(I)Br catalysis, 2,2'-bipyridine ligand and 2-bromoisobutyrate were purchased from Aldrich.

2.2 Characterization

Wide-angle X-ray diffraction (XRD) data were collected using MXP18V (Mac Science Co., Ltd.) at 40 kV, 200 mA. Transmission electron microscopic (TEM) observation was made on an LEO 922 (Carl Zeiss, Inc.) operated at 160 kV. Gel permeation chromatographic (GPC) analysis was carried out by a PU-2080 pump (JASCO Corporation) with a column (Shodex GPC SB-804HQ) and a differential refractometer RI-2031 (JASCO Corporation). Dynamic light scattering (DLS) measurements were performed by DLS-7000 (Otsuka Electronics Co., Ltd.) with Ar laser. ζ-potential measurement was performed by ELS-8000 (Otsuka Electronics Co., Ltd.). Ultraviolet-visible (UV-vis) absorption spectra were recorded by V-560 (JASCO Corporation).

2.3 Synthesis of magnetic particles

The magnetic nanoparticles were prepared according to a reported method [13]. The magnetic nanoparticles were obtained as black powder.

2.4 Synthesis of surface initiator.

The surface initiator was synthesized according to a reported reference [14]. The designed surface initiator has both bromine atom that is a living ATRP moiety and a phosphoric acid moiety that can interact with magnetic nanoparticle surface.

2.5 Immobilization of surface nitiator on magnetic nanoparticles

The designed surface initiator has both a living ATRP moiety and a phosphoric acid moiety that can interact with magnetic nanoparticle surface. Immobilization of surface initiator onto the surface was conducted as follows. Magnetic nanoparticles and initiator (1:1, w/w) were added to methanol, and the suspension was sonicated for 30 min. After cooling, centrifuged (5000 rpm 30 min), decanted, and added with methanol, the suspension was sonicated again. The cycle performed four times to remove the non-chemisorbed initator. After dried under reduced pressure, surface initiator coated magnetic nanoparticles were obtained.

2.6 Synthesis of biomolecules with pH response, bioaffinity, and protein immobilization functionality on magnetic nanoparticles surface

DEA monomer was used as pH response ability. MPC monomer was used as bioaffinity ability. Block copolymer composed of MPC and MEONP was synthesized as bioconjugate interface. The surfaces covered with MPC that can inhibit the non-specific protein adsorption were located inside of grafted polymer to defense non-specific protein adsorption at near of magnetic core. Typical protocols using ATRP for surface initiator coated Fe_3O_4 nanoparticles were as follows. First of all, monomer and ethyl 2-bromoisobutyrate as a free initiator was dissolved in 10 mL methanol. Free initiator was used as control ratio of monomer to initiator and to estimate molecular weight. The surface initiator coated magnetic nanoparticles were added to the solution and then dispersed by sonication. After bubbling with Argon for 15 min, the Cu(I)Br catalyst and 2,2'-bipyridine ligand were added to the stirred solution under Ar. The relative molar ratios of [free initiator]:[Cu(I)Br]:[bpy] were 1:1:2 for monomer concentration. In the case of polymerization of DEA and MPC monomer, above method was used. In the case of MPC-*b*-MEONP block copolymer synthesis, when conversion of MPC monomer reached 99 %, degassed 10mL methanol with dissolved MEONP monomer was added polymer solution. In order to stop reaction, the polymer solution was exposed to air and then passed throw silica gel column to remove Cu catalyst. The polymer grafted-magnetic nanoparticles were separated by centrifugation under 13000 rpm for 30 min x 3 cycles. The supernatant was evaporated and freeze dried to obtain solid state polymer.

2.7 Dispersion property of pH response and bioaffinity polymer grafted magnetic nanoparticles

The pH response polymer-grafted magnetic nanoparticles surfaces were dispersed in various pH solutions by sonication. The pH was prepared by HCl or NaOH aqueous solution. The diameters of nanoparticles in each pH solutions were evaluated by DLS. The pMPC and pMPC-*b*-MEONP block copolymer grafted magnetic nanoparticles were also dispersed in methanol, ethanol, PBS buffer solution, THF, acetone. The concentrations of particles were prepared 0.1 mg/mL. To investigate dispersion property on polymer grafted magnetic nanoparticles in detail, ζ-potential was measured in PBS buffer solution.

2.8 Immobilization of protein on magnetic nanoparticles surface

Protein, bovin serum albumin (BSA) was dissolved in PBS (pH 7.4) and the concentration was prepared 0.1 mg/mL. pMPC-*b*-MEONP-grafted magnetic nanoparticle dispersed solution (0.1 mg/ml) in the PBS solution. The mixed suspension was kept in the refrigerator and stirred with a magnetic stirrer for 48 h. The BSA was immobilized with the surface of magnetic nanoparticles throw condensation between the active ester unit of MEONP and amino group of BSA. After immobilization, the mixed suspension was centrifuged at 13000rpm for 30 min at 4 °C. The amount of released *p*-nitrophenol was estimated compared to analytical curvat 400 nm by UV-vis spectrum measurement e. To check initial amount of *p*-nitrophenol on surface of magnetic nanoparticle, hydrolysis of the *p*-nitrophenyl ester was performed by adding 3 drops of 0.2 M NaOH aqueous solution. To estimate amount of immobilized BSA, micro BCA protocol was performed after washed and removed unreacted BSA.

3. RESULTS AND DISCUSSION

3.1 Characterization of magnetic nanoparticles

Fig.2 shows TEM images and XRD patterns of synthesized particles. From TEM images, magnetic nanoparticles with diameter about 10-15 nm were observed. It was also confirmed that the nanoparticles were magnetite from XRD pattern.

3.2 Characterization of polymer-grafted magnetic nanoparticles

Before characterization of polymer-grafted magnetic nanoparticles, immobilization of surface-initiator on

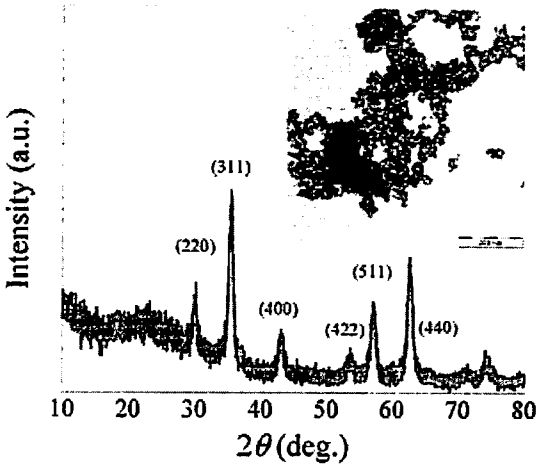

Fig.2 TEM images and XRD patterns of synthesized particles. Bar indicates 200 nm.

magnetic nanoparticles was conformed by FT-IR spectrum measurement (data not shown here). Table I summarized representative polymer, polymerization condition, molecular weight, and polydispersities. The evaluated molecular weight and target molecular weight were not in agreement because PEG was used as a standard polymer. On the other hand, the polydispersivity were relative narrow.

3.3 Dispersion property of polymer-grafted magnetic nanoparticles in various solvents

Fig.3 shows diameter of pDEA-grafted Fe_3O_4 nanoparticles evaluated by DLS in various pH solution. The diameters of nanoparticles in each pH solutions were depended on pH. At pH 9.5, pDEA-grafted Fe_3O_4 nanoparticles were dispersed temporarily by sonication with larger diameter. However, they aggregated after a few hours. In contrast, at below pH 6.2, they continued to disperse at least over two month.

Fig.4 indicates ζ-potential of pDEA-, pMPC- and pMPC-b-MEONP-grafted Fe_3O_4 nanoparticles. The pH response polymer brush, pDEA grafted-magnetic nanoparticles can be dispersed in below pKa 7.3 aqueous solution due to protonation overcome magnetic interaction. Therefore, compositions of the block polymer containing DEA have the possibility of construction of the pH response surface.

On the other hand, pMPC and pMPC-*b*-MEONP grafted-nanoparticles can be dispersed in neutral

Table I. Summary of molecular weight date for representative polymer [a]

entry	Target composition	time (h)	conversion(%)[b]	M_n[c]	M_w/M_n[c]
1	DEA_{50}	24	>99	3400	1.43
2	MPC_{30}	3.5	91	5300	1.28
3	MPC_{100}-*b*-$MEONP_{15}$	48	-	8200	1.30
	initial MPC_{100}	10	>99	7500	1.33

[a]:The relative molar ratio of Free initiator:CuBr:bpy=1:1:2, and methanol 10 mL with Initiator-coated magnetic nanoparticles 10 mg for all syntheses. Temperature at 60°C for DEA, at 20°C for MPC and block copolymer.

[b]:As determined by ^{1}H-NMR spectroscopy.

[c]:As determined by GPC using PEG standard, MeOH/H_2O (7/3 v/v) with 0.2M LiBr eluent,0.4 mL/min.

aqueous solution. They were not related to pH. Generally, colloid particles stabilized due to balance of electrostatic interaction in aqueous solution. In the case of pDEA-grafted Fe_3O_4 nanoparticles, an electrostatic interaction is not suitable for the reason of dispersion in the study of dispersibility because pDEA is cationic polymer shown from ζ-potential. On the other hand, pMPC and pMPC-*b*-MEONP-grafted nanoparticles showed neutral charge. Therefore, the nanoparticles did not be dispersed due to electrostatic interaction in aqueous solution. It is considered that steric repulsion of surface polymer brush most influenced the dispersibility. Although it is not relate to charge of surface, the nanoparticles can be also dispersed in methanol, ethanol for good solvent while they could not be dispersed in THF and acetone for poor solvents. That is, the dispersion of nanoparticles can be changed over solubility for surface grafted polymer.

3.4 Estimation of immobilization of protein on magnetic nanoparticles surface

pMPC-*b*-MEONP-grafted magnetic nanoparticle was used as protein immobilization bead. Total amount of *p*-nitrophenol was 5.3 x 10^{-4} ± 0.2 mg/mL before immobilization of BSA from UV-vis spectra measurement. Thereby, *p*-Nitrophenol molar per 1mg was estimated at 3.8 x 10^{-5} ± 0.2 mmol/mg by using *p*-nitrophenol molecular weight Fw:139. After immobilization of BSA, the concentration of BSA on nanoparticles was estimated at 2.0 x 10^{-3} ± 1.3 mg/mL and BSA molar per 1mg was 3.0 x10^{-7} ± 1.3 mmol/mg by using *p*-nitrophenol molecular weight 66500. It is concluded that bioaffinity polymer with protein immobilization part, MEONP, grafted nanoparticles can capture proteins with high probability.

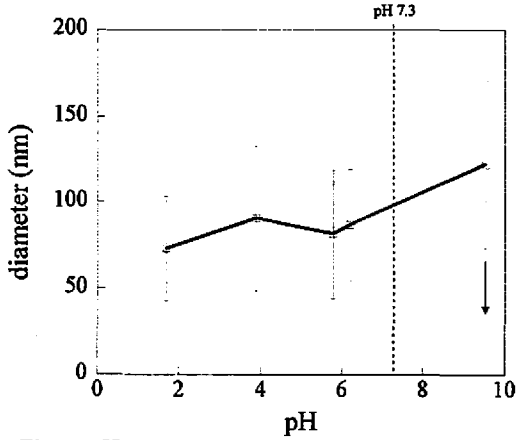

Fig.3 pH-dependent diameter of pDEA-grafted Fe_3O_4 nanoparticles. An arrow indicates after a few hours.

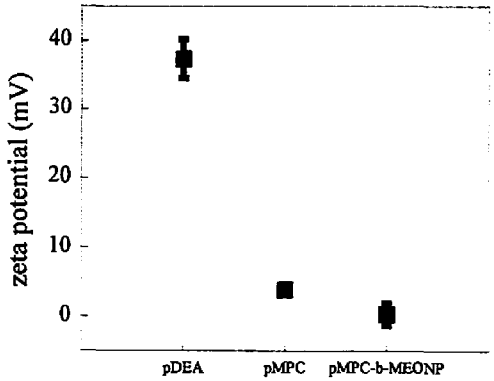

Fig.4 ζ-potential of pDEA-, pMPC- and pMPC-*b*-MEONP-grafted Fe_3O_4 nanoparticles. pH3.81 PBS solution for pDEA grafted-Fe_3O_4, PBS solution for pMPC and pMPC-*b*-MEONP grafted-Fe_3O_4.

4. CONCLUSIONS

In this study, biomolecules with pH response, bioaffinity and protein immobilization containing *p*-nitrophenyl ester groups was synthesized from surface initiator coated on magnetic nanoparticles by ATRP. The dispersion of polymer-grafted nanoparticles can be changed over solubility for surface grafted polymer. The BSA was immobilized on the surface of the nanoparticles. Bioaffinity polymer with protein immobilization part grafted nanoparticles synthesized by ATPR will be a useful for various bioaffinity bead applications.

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