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Framework effect of amphiphilic polyesters on their molecular movement and protein adsorption-resistance properties

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ABSTRACT

Surface chemical characteristics of biomedical polymers, which are determined by the migration and rearrangement of polymeric chains, play an important role in the protein adsorption. In this work, the relationship between the architectures of amphiphilic polyesters and their protein adsorption resistance was investigated. Three poly (ε-caprolactone)s containing sulfobetaines (PCL-b-PDEAS) segments with linear, four arms and six arms star-shaped architectures were synthesized with the combination of ring-opening polymerization (ROP) and atom transfer radical polymerization (ATRP). The structures of the amphiphiles were confirmed by ^{1}H NMR and FTIR. Water contact angles (WCA) and X-ray photoelectron spectroscopy (XPS) were used to study the surface properties of the amphiphilic copolymer films. The water contact angles were decreased due to the surface migration of hydrophilic segments. Transmission electron microscopy (TEM) displayed the occurrence of microphase separation phenomena for PCL-b-PDEAS above glass transition temperature (Tg). The results showed that the hydrophilic segments in the copolymers would migrate to the surface of the films, which resulted in the surface more hydrophilic to resist protein adsorption. The adsorption of both fibrinogen (Fg) and bovine serum albumin (BSA) were studied. The results showed that protein adsorption was depended on not only the hydrophilic chain migration but also the shape of proteins.

1. Introduction

Surface properties are very important to biomedical polymers as complicated reactions such as thrombosis, immune activation and reject reaction between human bodies and biomedical polymers occurred on the surface of biomedical polymers. To obtain biomedical polymers with desirable surface properties has become one key point in the design and synthesis of biomedical polymers. Once the polymers contact with body fluid or blood, the first step is the protein adsorption on the surface, and then other chain reactions such as immunological reaction are activated, thus, non-protein adsorption surface is favorable for the applications of biomedical polymers. Surface modification is a useful and convenient approach to achieve polymeric surfaces with specific property. Improvement of surface hydrophilicity is considered as one of the most important strategies to prevent the protein adsorption [1–6]. Many hydrophilic polymers including PEG, albumin, heparin, and phospholipids were introduced in biomedical polymers to increase the surface hydrophilicity [7–9]. Poly (ethylene glycol) (PEG) has exhibited the exciting resistance in protein adsorption due to the "steric repulsion" and hydration layer formed via hydrogen bonding around molecular chains.

Besides PEG, zwitterionic sulfobetaines have aroused great attention for their water solubility and excellent biocompatibility featured by non-fouling and protein-resistance properties. Studies have demonstrated that the surfaces of poly (ether urethane), low-density polyethylene and cellulose grafted with zwitterionic sulfobetaines could effectively suppress protein adsorption and denaturation even in contact with whole blood [10–12]. Therefore, to introduce zwitterionic sulfobetaine groups in polymers has attracted much interest to biomaterials scientists to receive non-protein adsorption surfaces. The low protein adsorption for the polymer surfaces containing zwitterionic sulfobetaines

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is attributed to the solvation of the charged terminal groups besides hydrogen bonds [13,14]. Further studies discover that the non-protein adsorption ability of the sulfobetaine-based polymers is also depended on their packing density, which in turn affects the hydration of sulfobetaine chains. It is clearly noted that prior researches typically revolve around the development of sulfobetaine units grafted on the surfaces of materials with the idea of reducing the potential chemical interactions between blood and surfaces of polymers.

As soft materials, polymer surfaces are dynamic and would reorient to minimize surface free energy in response to environmental changes. Once sulfobetaine units are introduced into polyester or polyurethane bulks, the sulfobetaine segments would migrate to the polymer surface to form a more thermodynamically favorable interface when exposed to water, and this rearranged surface would lower the protein adsorption. The surface migration phenomenon was observed in other water soluble phosphorylcholine groups in poly(2-methacryloyloxyethyl phosphorylcholine)-co-poly(n-butyl methacrylate) and poly(6-methacryloyloxyhexyl phosphorylcholine)-co-poly(n-butyl methacrylate) when contacted with water. The surface composition of amphiphilic copolymer would reorganize to decrease the surface energy in response to contacting media. Typically, hydrophilic moieties migrated to the surface to lead the surface more hydrophilic in water medium [4,15,16]. However, the researches about the surface composition rearrangement of amphiphilic polymers were nearly limited in linear polymers.

The star-shaped polymers have exhibited different characteristics to linear polymers, such as less entanglement in the solid state, high solubility in various solvents and fast molecular motion [17–19]. Xu et al. [20] has demonstrated that star-shape SPHBCL block copolymers have presented better biocompatibility than linear copolymers. Other studies have confirmed that the architecture of copolymer exerts effects on the biocompatibility of copolymers, such as protein adsorption, platelet adhesion and so on [21]. Therefore, the aim of this work was to explore the difference of surface migration of sulfobetaine units between linear and star-shaped poly(ε-caprolactone)-b-poly(N,N-diethylaminoethyl methacrylate) sulfobetaine copolymers, the protein adsorption behavior on the surface of linear/star-shaped amphiphilic copolymers was studied. The copolymers were synthesized via the combination of ring-opening polymerization (ROP) and atom transfer radical polymerization (ATRP) (Scheme 1). The surface reorganization kinetics of the amphiphilic copolymers were characterized by water contact angle (WCA), X-ray photoelectron spectroscopy (XPS) and transmission electron microscopy (TEM). The relationship between surface chemical characteristics of star-shaped copolymers and protein adsorption was discussed.

2. Materials and methods

2.1. Materials

N,N-diethylaminoethyl methacrylate (DEA) (Aldrich, USA) and ε-caprolactone (CL) (New Jersey, USA) were dried in calcium hydride (CaH_2) (Chengdu Kelong Chemicals Ltd., China) and distilled before used. Toluene and tetrahydrofuran (THF) (Chengdu Kelong Chemicals Ltd, China) were dried with sodium and refluxed before used. Stannous octoate ($Sn(Oct)_2$), 2-bromoisobutyryl bromide, copper (I) bromide (CuBr, 99.999%), 2,2′-bipyredine (bpy), dodecanol, pentaerythritol, dipentaerythritol, fibrinogen (Fg), albumin (BSA) were purchased from sigma (St. Louis, MO, USA) and used as received. 1,3-propanesultone (PS) was supplied by Acros and used as received. All other reagents and solvents were analytical grade and used as received.

2.2. Synthesis of linear and star-shaped poly (ε-caprolactone) (L/sPCL-OH)

Linear and star-shaped PCLs with four or six arms were prepared by ring opening polymerization (ROP) with dodecanol as initiator, pentaerythritol and dipentaerythritol at 120 °C, respectively. $Sn(Oct)_2$ was used as catalyst. The synthesis of six-arm star-shaped PCL (6sPCL-OH) was used as an example to show the synthetic process. Typically, ε-CL (1.0 g, 8.8 mmol), dipentaerythritol (0.0186 g, 0.07 mmol) and a magnetic flea were put into a dry polymerization tube and $Sn(Oct)_2$ (1 mg, wt. 0.1%) in dry toluene was added. After purged with nitrogen for three times to remove the residue toluene and trace water, the tube was put into an oil bath at 120 °C for 24 h. The product was dissolved in dichloromethane (CH_2Cl_2) and precipitate in cold methanol. The precipitate was filtered and vacuum-dried to constant weight. Yield: 94.3 wt.%.

2.3. Synthesis of L/sPCL-Br macroinitiators

6sPCL-OH (1.0 g, 0.072 mmol) was added into a Schlenk flask. Dry toluene (10 mL) and triethylamine were added under nitrogen atomosphere. After the copolymer was dissolved completely, the flask was placed into an ice salt water bath (−20 °C), 2-bromoisobutyryl bromide (79.9 μL, 0.646 mmol, 9 equiv) was added with a rate of 10 μL/min. The mixture was warmed to room temperature and stirred for 24 h. The insoluble salt was removed by filtration. The filtrate was condensed and precipitated in cold methanol. The product was dried in vacuum overnight to constant weight. Yield: 82.20 wt.%.

2.4. Synthesis of L/sPCL-b-PDEA block copolymers

L/sPCL-b-PDEA copolymers were synthesized via atom transfer radical polymerization (ATRP). The synthesis of 6sPCL-b-PDEA copolymer was used as an example. A Schlenk flask contained a magnetic stirring bar and 6sPCL-Br macroinitiator (1 g, 0.069 mmol) was connected to a Schlenk line, the flask was purged with nitrogen for three times, anhydrous CH_2Cl_2 (10 mL) was added in the flask. After the macroinitiator was completely dissolved, dry DEA monomer (1.548 g, 8.323 mmol, 120 equiv) was added under nitrogen atmosphere. Cu(I)Br (0.0755 g, 0.526 mmol, 7.2 equiv) and bpy ligand (0.1644 g, 1 mmol, 14.4 equiv) were added in the mixture under nitrogen atmosphere. The reaction was conducted for 48 h under at room temperature. The solution was diluted with THF, purified with a neutral Al_2O_3 column and precipitated into hexane. The copolymer was dried in vacuum to constant weight. Yield: 70.2 wt.%.

2.5. Synthesis of sulfobetainized copolymers (L/sPCL-b-PDEAS)

6sPCL-b-PDEA (1.0 g, 0.0448 mmol) and PS (0.787 g, 6.452 mmol, 1.2 equiv) were dissolved in dry THF (20 mL) in a 100-mL round-bottom flask equipped with a stirring bar and stirred at *room* temperature. After 6 days, the solution was dialyzed against ethanol for 3 days and further against distilled water for 2 days. A light yellow product was obtained after lyophilized. Yield: 80.2 wt.%.

2.6. Characterization of L/sPCL-b-PDEAS copolymers

The ^{1}HNMR (400 MHz) spectra was performed on a Varian Inova NMR spectrometer. $CDCl_3$ with tetramethylsilane (TMS) as an internal standard and the mixture of $CDCl_3$ and CD_3OD were used as solvents. The attenuated total reflection Fourier Transform Infrared (ATR-FTIR) spectra were recorder on a Nicolet Magna 560 FTIR spectrometer in the range between 4000 and 500 cm^{-1} with a resolution