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Poly(ε-caprolactone) modification via surface initiated atom transfer radical polymerization with bio-inspired phosphorylcholine

Liu He [a], Lei Huang [a], Songbai Zhang [a], Yuanwei Chen [a,*], Xianglin Luo [a,b,**]

[a] College of Polymer Science and Engineering of Sichuan University, Sichuan University, Sichuan 610065; PR China
[b] State Key Lab of Polymer Materials Engineering, Sichuan University, Sichuan 610065, PR China

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ABSTRACT

Bio-inspired phosphorylcholine modification on material surface has shown great promise in constructing biocompatible materials. In this study, poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) was grown on poly(ε-caprolactone) (PCL) surface in order to suppress protein adsorption and cells adhesion and to improve blood compatibility. The initiator for surface initiated atom transfer radical polymerization (SI-ATRP) was covalently tethered on PCL surface and then PMPC brushes with diverse graft amounts were grafted to PCL film. X-ray photoelectron spectroscopy (XPS) and water contact angle measurement were used to characterize the modified sheets. The PMPC-grafted PCL sheets showed lower protein adsorption, maintained secondary structure of detached protein, and suppressed adhesion and pseudopodium formation of the platelets, along with keeping longer activated partial thromboplastin time (APTT) in comparison with PCL membranes. At the same time, PMPC-grafted PCL sheets suppressed the LO2 cells adhesion. These results showed that phosphorylcholine SI-ATRP modification on PCL surface may provide PCL more biocompatible in biomaterial applications.

1. Introduction

Biomaterials need to have good biocompatibility, for example, the materials contacting with blood require excellent blood compatibility, meanwhile, implant material contacting with tissues must have good histocompatibility. These properties depend on the protein adsorption layer on material surface. Protein adsorption on the surface of various materials is a very common phenomenon. The non-specific protein adsorption on biomaterials is undesired. The adsorption of fibrinogen on blood contact biomaterials may induce thrombosis, and non-specific protein adsorption on implanted material will induce adhesion of bacteria or macrophage then cause inflammation.

Over the past decades, many polymers, like polyethylene glycol (PEG) [1–4], and zwitterionic polymers [5–7], are used to suppress non-specific protein adsorption on biomaterial surfaces to improve the biocompatibility of substrates or devices. However, PEG is unstable and will decompose into small fragments in the condition of oxygen and transition metal ions which are found in most biochemically relevant solutions [8,9]. Thus, zwitterionic polymers with special biomimetic nature are showing charm in the biomedical applications for their particular characteristics such as long-term circulation which is required for drug delivery carriers, stability and resistance of non-specific protein adsorption requested for implant materials. Kdilk.

Among numerous zwitterionic polymers, PMPC formed by 2-methacryloyloxyethyl phosphorylcholine (MPC) with phospholipid groups (PC) which present in outside surface of the red blood cell membrane [10], shows excellent properties in resistance of protein adsorption and cell adhesion. MPC has been widely used as component of various biomaterials including micelle materials [11–13], polymer membranes [14–16] and metals [17,18] to enhance biocompatibility. Feng et al. grafted MPC by surface initiated atom transfer radical polymerization (SI-ATRP) on the surfaces of glass or silicon wafer and systematically investigated the effect of graft density and graft thickness of MPC on protein adsorption [19–22]. Besides grafting MPC to the surfaces of inorganic materials, MPC is also used to modify polymer surfaces to improve resistance of protein adsorption and lubrication. Xiong's group [15] modified ultrahigh molecular weight polyethylene (UHMWPE) by grafting MPC on the surface to enhance blood compatibility. Until now, there are few reports about the surface modification of poly(ε-caprolactone) (PCL) by MPC. As we know, Cai et al. synthesized copolymers of MPC and ε-caprolactone, and the membranes of the copolymers displayed resistance protein adsorption and antibacterial adhesion [14].

Here we used bio-inspired MPC to construct biocompatible surface of PCL by SI-ATRP. The initiator for SI-ATRP was introduced to surface through converting PCL surface group into amine and covalently linking 2-bromo-2-methylpropanoyl bromide on PCL surface, and finally PMPC

* Corresponding author.
** Correspondence to: X. Luo, College of Polymer Science and Engineering of Sichuan University, Sichuan University, Sichuan 610065, PR China.
E-mail addresses: chenyuanweizm@163.com (Y. Chen), luoxl@scu.edu.cn (X. Luo).

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chains were grown from PCL surface. The modified sheets were characterized by XPS and water contact angle. Protein adsorption, cell adhesion and activated partial thromboplastin times (APTT) were investigated to value the biocompatibility of the modified sheets and pristine sheets.

2. Experimental

2.1. Materials

Triethylamine (TEA) was dried by adding KOH before utilization. 2-bromoisobutyryl bromide (BIBB, 98%) was purchased from Sigma (USA). 1,6-hexanediamine was distilled under vacuum. Poly(ε-caprolactone) (PCL) pellets (Mw ~ 80,000) are the product of Shenzhen Esun Industrial Chemical Company. Tris(2-dimethylaminoethyl)amine (Me6TREN, 99%) was purchased from TCI. 2-methacryloyloxyethyl phosphorylcholine (MPC) was purchased from JOY-Nature Chemical Co. Micro BCA™ Protein Assay Reagent kits were purchased from Thermo Fisher Scientific Inc. Bovine serum albumin (BSA) and fibrinogen (Fg) are the products of Biosharp Inc. APTT and TT Reagent kits were purchased from SIEMENS.

2.2. Preparation of PMPC-grafted PCL sheets

The PCL (M_w ~ 80,000) sheets were prepared by hot compression above polymer melting temperature (80 °C) for 30 min and then equilibrated at room temperature. The pristine sheets were cut into specimen with the size of 1 cm × 1 cm and were washed with copious mixture of water/ethanol (1/1, v/v) [23], and then dried under vacuum.

The cleaned PCL sheets were subsequently immersed in 80 mg/mL 1,6-hexanediamine/2-propanol solution for 1 h at 37 °C to produce PCL-NH_2 sheets (as shown in Fig. 1). The PCL-NH_2 sheets were put into 35 mL of distilled hexane with 0.3 mL dried TEA, followed by adding 0.8 mL BIBB dropwisely. The reaction mixture was carried out at 0 °C for 2 h, and then additional 12 h at room temperature to produce the initiator-immobilized PCL sheets (PCL-Br). The PCL-Br sheets were washed successively with water, methanol and methanol/water (1/2, v/v) mixture and dried at 25 °C under vacuum.

Under the protection of nitrogen, MPC (500 mg, 1.69 mmol), $CuSO_4 \cdot 5H_2O$ (8 mg, 0.035 mmol), 9 μL Me_6TREN and 18 mL deionized water were added separately into a glass tube in which there were glass pricks on the bottom. The mixture was degassed by three pump-thaw cycles. After adding D-isoascorbate, PCL-Br sheets were added to the tube in erect state under nitrogen. The system was degassed finally by pump-thaw cycle three times. The reaction was allowed to proceed under darkness at room temperature. After predetermined period of time, the surface-modified sheets were moved out and washed with copious water and ethanol before dried under reduced pressure. The reaction time points for PMPC-grafted PCL sheets (PMPC-g PCL) were set to be 4 h (M-4), 8 h (M-8) and 24 h (M-24).

2.3. Surface components and hydrophilicity

The X-ray Photoelectron Spectroscopy (XPS) was used to characterize the components of PMPC-g PCL surfaces. It was performed in an AXIS ULTRA spectrometer (Shimadzu Co. Japan) using a monochromated Al Kα X-ray source and had the same procedure to the earlier literature [24]. Static water contact angle was used to evaluate the hydrophilicity of the modified plate surface, using the sessile drop method (KRUSS DSA 100).

2.4. Protein adsorption and secondary structure of the detached protein

Samples were placed in individual wells of a 24-well tissue culture plate. After equilibration by PBS (0.1 mol/L, pH = 7.4) for 1 h, the sheets were exposed to single protein solutions (1 mg/mL) of bovine serum albumin (BSA) and fibrinogen (Fg) for 3 h in a shaker at 37 °C, respectively. After rinsing the sheets with fresh PBS several times to remove loosely attached protein, the sheets were transferred to new wells and washed with 0.4 mL 0.1 wt% SDS aqueous solution at 37 °C for 30 min to extract adsorbed proteins from surfaces. Based on the principle of the bicinchoninic acid (BCA) protein assay kit method, the protein concentration in SDS solution was determined by a Microplate Reader, and the optical absorbance was recorded at a wavelength of 578 nm. The reported data was calculated from values of four parallel samples for each sheet.

The secondary structure of the detached protein from the surfaces was characterized by circular dichroism (CD) spectroscopy. Protein experimental samples were prepared by firstly immersing modified sheets into PBS for 24 h, and then transferred into 1 mL protein solution (20 mg/mL) with BSA and Fg respectively. After saturated adsorption at 37 °C for 2 h, the sheets were rinsed by PBS and water, and then the protein for CD test was detached using 400 μL 2 wt% SDS solution under slow vibration at 37 °C for 1 h. Protein control samples were 2 μg/mL BSA and 3 μg/mL Fg solution containing 2 wt% SDS and were slow oscillated at 37 °C for 1 h.

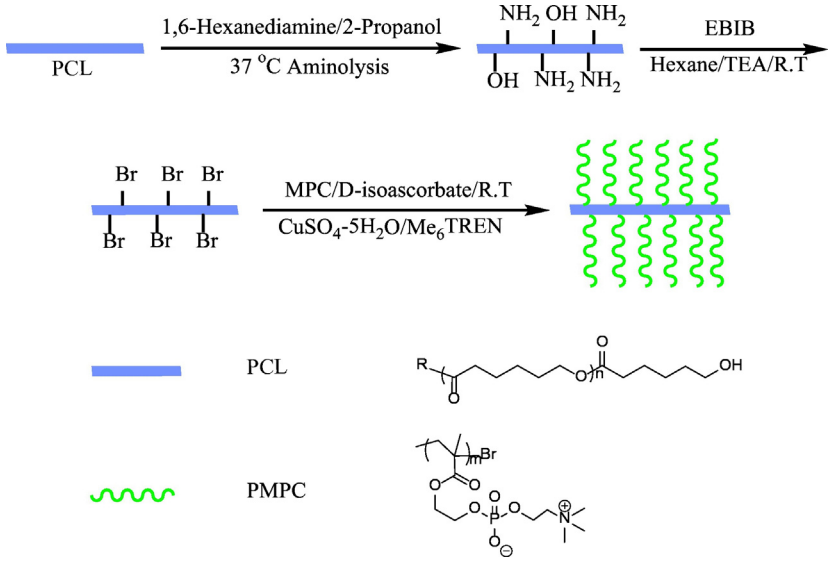

Fig. 1. Illustration of the surface-initiated ATRP of 2-methacryloyloxyethyl phosphorylcholine (MPC) from PCL-Br surface to produce the PMPC-g-PCL surface.