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Preparation of a chemically anchored phospholipid monolayer on an acrylated polymer substrate

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Abstract

This paper describes a strategy for designing a chemically anchored phospholipid monolayer that could be used as coating materials for biomedical implants. To make a chemically anchored phospholipid monolayer on the polymer substrate, we prepared the mono-acrylated phospholipid (1-palmitoyl-2-[12-(acryloyloxy)-dodecanoyl]-*sn*-glycero-3-phosphocholine; acryloyl-PC) and the acrylated polymer (poly(octadecylacrylate-*co*-4-acryloyloxy butylacrylate)), which was synthesized by the acrylation of poly(octadecyl acrylate-*co*-hydroxybutyl acrylate, poly(OA-*co*-HA)) with acryloyl chloride. The chemically anchored phospholipid monolayer was prepared by using in situ photopolymerization of a pre-assembled phospholipid monolayer, produced by lipid vesicle fusion, onto the acrylated polymer coated silicon wafer. Optimal condition of vesicle fusion and irradiation time was determined from the degree of hydrophilicity rendered by the polymerized phospholipid surface. The physicochemical properties of polymerized phospholipid monolayer on the substrate were evaluated using water contact angle, field-emission scanning electron micrograph (FE-SEM), atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS). These results confirmed that the polymerized phospholipid monolayer was chemically anchored on the acrylated polymer substrate. The chemically anchored phospholipid monolayer was stable in aqueous condition for 2 weeks, but the physically adsorbed phospholipid monolayer got removed within 1 day. Moreover, the polymerized phospholipid monolayer also suppressed albumin absorption and platelet adhesion, in vitro. This polymerized phospholipid monolayer provides a new biomimetic system for coating medical devises.

Keywords: Phospholipid monolayer; In situ photopolymerization; Acrylated polymer substrate; Blood compatibility

1. Introduction

Biomaterials are of great importance in biomedical applications, for example, in the implantation of artificial organs, artificial heart values, electrodes, catheters, and various other prosthetic devices into body. Unfortunately, biomaterials can induce adverse reactions such as the formation of thrombi due to deposition of blood proteins, which cause platelet adhesion, activation and the clotting cascade. These biomaterials also can stimulate undesirable immune responses such as proteolysis, cell lysis, opsonization, anaphylaxis, chemotaxis, etc., when they are placed in vivo [1]. Therefore, it is very crucial to develop biomaterials that must not stimulate changes in plasma proteins or cause the formation of thrombi and an immunologic reaction.

Considerable research effort has been devoted to the development of biomaterials for medical devices. Many research groups have utilized zwitterionic phosphorylcholine-modified surfaces in order to mimic the endothelial cell membrane since it is resistant to protein

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adsorption and cell adhesion and it can reduce the clot formation under physiological conditions [2–5]. In the past decade, a variety of polymers, such as polymethacrylates, polyurethanes, polydimethylsiloxanes, or polysulfones, have been designed as copolymers containing the phosphorylcholine moieties to produce nonthrombogenic and antifouling surfaces [3,6–9]. These phosphorylcholine-modified copolymers have suppressed protein adsorption and platelet adhesion, in vitro or in vivo. While these phosphorylcholine-modified copolymers with improved biocompatibility have been developed, none have completely mimicked the cell-membrane structure, which is composed mainly of highly dense phosphorylcholine surface.

On the other hand, self-assembled phospholipid monolayers and bilayers prepared by using Langmuir–Blodgett (LB) technique and vesicle fusion methods could offer highly dense phosphorylcholine surfaces on solid surfaces [10–15]. These phospholipid monolayers and bilayers on solids have proved to be useful for mimicking well oriented and highly packed phospholipid surfaces since the adsorption of proteins and blood cells to the zwitterionic phospholipid assemblies is much lower than the control surface [13,16,17]. However, supported phospholipid membranes have an obvious drawback of limited stability since the physically adsorbed phospholipids are easily solubilized and washed out in surfactants and organic solvents. Furthermore, the physically adsorbed phospholipid monolayers are removed from the surface during sterilization process, such as autoclave and ethylene oxide gas sterilization.

To increase the stability of phospholipid monolayers, we have reported a method to prepare a chemically anchored phospholipid monolayer using in situ polymerization at the interface between a mono-acrylated phospholipid monolayer and a methacryloyl-terminated surface [18–20]. The phospholipid monolayer, chemically anchored to solid substrates, formed an oriented and packed monolayer structure with a high anchoring coverage, above 87%. The chemically anchored phospholipid monolayer on a solid substrate was very stable in surfactant and organic solvent, as reported in our earlier work [18]. Moreover, this polymerized phospholipid surface prevented protein adsorption and platelet adhesion, in vitro and it suppress immune reaction, in vivo [19]. However, the chemically anchored phospholipid monolayer has limited application for the surface modification of biomaterials, because it could be applied only to the surface containing polymerizable moieties, which can induce covalent bonding to the pre-assembled and mono-acrylated phospholipids monolayer during in situ polymerization. Thus, the chemically anchored phospholipid monolayer was difficult to be widely applied to polymeric and metallic medical devices without polymerizable moieties on their surfaces, for example, coronary guidewires, stents, and extracorporeal circuits, etc.

In this study, we tried to overcome this limited application of chemically anchored phospholipid monolayer by synthesizing an acrylated polymer (poly(octadecylacrylate-*co*-4-acryloyloxy butylacrylate)) that could be easily coated on various polymeric and medical devices by using a dip coating or spin coating method. First, the silicon wafer surface was spin-coated with the acrylated polymer (the acrylated polymer substrate), followed by assembling of mono-acrylated phospholipid monolayers onto the acrylated polymer substrate. And, the chemically anchored phospholipid monolayer was produced by in situ photopolymerization of the pre-assembled mono-acrylated phospholipid monolayer onto the acrylated polymer substrate. Physicochemical properties of anchored phospholipid monolayer on the acrylated polymer substrate and its in vitro blood compatibility were evaluated.

2. Materials and methods

2.1. Materials

Octadecyl acrylate (OA), 4-hydroxybutyl acrylate (HA), 2,2′-azo-bis-isobutyrylnitrile (AIBN), chloroform, toluene, tetrahydrofuran (THF) and ethanol were analytical grade and purchased form Aldrich Chemical Co. (Milwaukee, WI). Eosin Y (EY; 5% in water), triethanolamine (TEA), 1-vinyl-2-pyrrolidinone (VP) and acryloyl chloride were obtained from Sigma Chemical Co. (St. Louis, MO). The mono-acrylated phospholipid, 1-palmitoyl-2-[12-(acryloyloxy)dodecanoyl]-*sn*-glycero-3-phosphocholine (acryloyl-PC), was synthesized as described in our previous study [18]. Bovine serum albumin (BSA, 99%) and FITC-labeled BSA (FITC-BSA, 99%) were purchased from Sigma Chemical Co.

2.2. Synthesis of acrylated polymer substrate (poly(octadecylacrylate-co-4-acryloyloxy butylacrylate))

For making polymerizable moieties on polymer surface, we first synthesized poly(OA-*co*-HA), and then hydroxy groups in side chains of poly(OA-*co*-HA) were acrylated with acryloyl chloride, as shown in Fig. 1. OA (1.0 g, 3 mmol) and HA (433 mg, 3 mmol) dissolved in 5 ml toluene were reacted in the presence of AIBN (16.8 mg, 0.06 mmol) at 70 °C for 24 h under argon environment. The reacted solution was cooled at room temperature and precipitated in 50 ml of ethanol. The product, poly(OA-*co*-HA), was precipitated and dried. The composition ratio of OA and HA was determined using ^{1}H NMR.