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Photo-assisted generation of phospholipid polymer substrates for regiospecific protein conjugation and control of cell adhesion ☆

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ABSTRACT

Novel photo-reactive phospholipid polymers were synthesized for use in the preparation of nonfouling surfaces with protein conjugation capacity. Poly[2-methacryloyloxyethyl phosphorylcholine (MPC)-*ran*-*N*-methacryloyl-(L)-tyrosinemethylester (MAT)] (P(MPC/MAT)) was synthesized by conventional radical polymerization, with the MAT units capable of being oxidized by 254 nm UV irradiation. Because of this photo-oxidation, active species such as catechol and quinone were alternately generated in the copolymer. A silicon wafer was subjected to surface modification through spin coating of P(MPC/MAT) from an aqueous solution for use as a model substrate. The surface was then irradiated several times with UV light. The thickness of the polymer layers formed on the Si wafers was influenced by various parameters such as polymer concentration, UV irradiation time, and composition of the MAT units in P(MPC/MAT). Oxidized MAT units were advantageous not only for polymer adhesion to a solid surface but also for protein conjugation with the adhered polymers. The amount of protein immobilized on UV-irradiated P(MPC/MAT) was dependent on the composition of the MAT units in the polymer. Furthermore, it was confirmed that protein immobilization on the polymer occurred through the oxidized MAT units because the protein adsorption was significantly reduced upon blocking these units through pretreatment with glycine. Conjugation of regiospecific protein could also be achieved through the use of a photomask. In addition, nonspecific protein adsorption was reduced on the non-irradiated regions whose surface was covered with physisorbed P(MPC/MAT). Therefore, P(MPC/MAT) can be used in the preparation of nonfouling substrates, which enable micrometer-sized manipulation of proteins through photo-irradiation. Function of proteins immobilized on MPC copolymers was also confirmed by cell adhesion test. As such, photo-reactive MPC copolymers are suitable for performing controlled protein conjugation and preparing polymer–protein hybrid platforms for use in biomedical and diagnostic devices.

Statement of Significance

Novel photo-reactive phospholipid polymers have been synthesized for immobilization on solid surfaces and regiospecific protein conjugation. Tyrosine residues embedded in 2-methacryloyloxyethyl phosphorylcholine (MPC) copolymers could be photo-oxidized, resulting in polymers able to form layers on a solid surface and conjugate with proteins. Moreover, nonspecific biofouling on the surface significantly reduced when the oxidized tyrosine units in the polymer layers were blocked. Upon UV irradiation through a photomask, the UV-exposed tyrosine units were selectively oxidized, forming the only specific regions in which protein conjugation could occur.

1. Introduction

Control of protein adsorption on synthetic materials remains an important issue for the development of biomedical materials [1–3]. Nonspecific protein adsorption can trigger unfavorable host responses, such as blood coagulation [4,5], inflammation [6–8], and infection [9,10], and can also cause reduction in the

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function of biomedical and diagnostic devices [11,12]. In contrast, specific molecular recognition can result in beneficial biological events for tissue engineering, separation, and biosensor applications [13]. We have determined that zwitterionic 2–methacryloyloxyethyl phosphorylcholine (MPC) polymers are suitable materials for such requirements [14]. In general, MPC polymer surfaces do not interact with plasma proteins because MPC units possess a zero zeta potential, hydrophilic nature, and high free-water content [15]. Moreover, the MPC polymer surfaces do not induce denaturation of proteins or activation of blood cells upon contact [15,16]. Upon addition of ligands to MPC polymer surfaces, specific molecular interactions between the surface and proteins or cells can be regulated [17,18]. Several ligands, such as nucleic acids [19], carbohydrates [20], peptides [21], and proteins [22] have been immobilized on MPC polymer surfaces. We previously introduced lactose residues on MPC polymer surfaces and found that galactose-binding lectin (RCA_{120}) and hepatocytes having asialoglycoprotein receptors selectively bound to the surfaces [20]. Ishihara et al. prepared MPC polymers bearing active esters and demonstrated the conjugation of enzymes [23] and antibodies [22,24], which they subsequently applied to diagnostic and drug delivery systems [25]. Although the conjugation of biomolecules on MPC polymer surfaces is robust enough to obtain biointeractive materials, reports describing user-defined and on-demand immobilization of biomolecules are still limited.

In the present study, novel photo-reactive MPC polymers bearing tyrosine units were synthesized. The oxidation of tyrosine has been well studied and is of interest in both biochemistry and engineering [26] as it can be oxidized with UV light [27], $H_2O_2/CuCl_2$ [28], and tyrosinase [29]. The oxidation products of tyrosine are relatively complex, with the proposed products including catechol, quinone, and dityrosine [27,28,30,31]. Pioneering work by Jin et al. studied the photolysis of tyrosine in aqueous media using 254 nm UV light and the effect of O_2 was determined [27]. 3,4-Dihydroxyphenylalanine (DOPA) was generated in the presence of oxygen as one of the most dominant O_2-dependent products.

Recently, DOPA has gained interest as a mussel-inspired adhesive amino acid, as the catechol of DOPA functions as a surface anchor [32]. Similarly, dopamine (DA), having both catechol and amine functionalities, adheres well to many kinds of substrates, including polymers, glass, and metals [33]. Binding mechanisms of catechol have been proposed to occur through covalent bonding, hydrogen bonding, and coordination, which are dependent on the surface functionalities of the substrate [32]. Various surface modification techniques have been explored for DA systems [34–37], with one of the most successful applications being the creation of nonfouling surfaces [38–40]. Furthermore, it has been reported that catechol and quinone states are in equilibrium in aqueous media [41] and that quinone can react with thiol and amine groups through a Michael addition reaction [41–44]. Therefore, protein immobilization on polydopamine (PDA) has been adapted for various solid surfaces.

The tyrosine units incorporated in MPC copolymers synthesized in the present study were oxidized by UV irradiation, generating active species such as catechol and quinone in the copolymers. A coating layer of MPC copolymers bearing tyrosine residues was formed on solid substrates via UV irradiation through binding of oxidized tyrosine residues on the substrate. This resulted in improvement in the nonfouling property of the substrates. Moreover, protein immobilization was performed through oxidized tyrosine units on the MPC copolymers with high tyrosine composition (~30 mol%). Regiospecific protein conjugation and controlled cell adhesion were also achieved.

2. Materials and methods

2.1. Chemicals

Si wafers (100 orientation, P/B-doped) were purchased from Yamanaka Semiconductor Co., Ltd., Tokyo, Japan. 2-Methacryloyloxyethyl phosphorylcholine (MPC) was kindly provided by NOF Co., Ltd. and used without further purification. *N*-Methacryloyl-(L)-tyrosine methyl ester (MAT) was synthesized via reaction of methacryloyl chloride and L-tyrosine methyl ester hydrochloride [45,46]. Other commercially available reagents and solvents were purchased in extra-pure grade and used without further purification.

Titanium, Copper, molybdenum, niobium, and Stainless (Nilaco, Tokyo, Japan), polyethylene (PE; Hitachi Chemical Co., Ltd., Tokyo Japan), polyethylene terephthalate (PET; Wako Pure Chemical Industries, Ltd., Osaka, Japan), polycarbonate (PC; Takiron Co., Ltd., Tokyo Japan), polyether ether ketone (PEEK; Röchling, Mannheim, Germany), polymethyl methacrylate (PMMA; Kuraray Co., Ltd., Tokyo, Japan), polytetrafluoroethylene (PTFE; Nippon Valqua Industries, Ltd., Tokyo, Japan) were cleaned ultrasonically in an appropriate solvents for ten min before use.

2.2. Synthesis of MPC copolymers

Poly(MPC) (PMPC) and MPC copolymers with MAT (P(MPC/MAT), Fig. 1) were synthesized by conventional radical polymerization using 2,2′-azobisisobutyronitrile (AIBN) as an initiator. The mole fraction of monomer units in the copolymers was determined by ^{1}H NMR spectroscopy. The apparent molecular weights of the MPC polymers were determined by gel permeation chromatography (GPC) using a Jasco GPC system equipped with a refractive index detector and size-exclusion columns, Shodex, SB-803 HQ, and SB-806M, with a poly(ethylene glycol) (PEG, Tosoh standard sample) standard in distilled water containing 10 mM LiBr. The results of polymerization are summarized in Table 1.

2.3. Photooxidation of MPC copolymers

Tris buffers were prepared by mixing appropriate quantities of 10 mM Trizma base and 10 mM Trizma HCl and adjusting their pH to 6.7, 7.4, and 8.5. P(MPC/MAT)70 whose number represents mol% of MPC in the feed and PMPC were dissolved in the buffer solutions and their concentrations were adjusted to 0.1 wt%. The polymer solutions were then exposed to UV light (254 nm) for

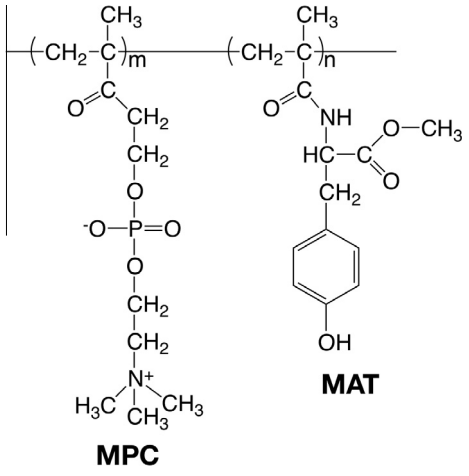

Fig. 1. Chemical structure of P(MPC/MAT).

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