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Research Article

Nano-structural comparison of 2-methacryloyloxyethyl phosphorylcholine- and ethylene glycol-based surface modification for preventing protein and cell adhesion

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ABSTRACT

Polymer brush, owing to its precisely controllable nanostructure, has great potential for surface modification in the biomedical field. In this study, we evaluated the bio-inertness of polymer brush, monomer monolayers, and polymer-coated surfaces based on their structures, to identify the most effective bio-inert modification. We focused on two well-known bio-inert materials, 2-methacryloyloxyethyl phosphorylcholine (MPC) and ethylene glycol (EG). The amount of adsorbed proteins on the surface was found to be dependent on the monomer unit density in the case of MPC, whereas this correlation was not observed in the case of EG. Cell adhesion was suppressed on the brush structure of both MPC and EG units, regardless of their density. The brush structure of MPC and EG units showed better anti-protein- and anti-cell-adhesion than monolayers and polymer-coated surfaces. Thus, the steric repulsion was not only important in EG units-based surface, but also in MPC-based surface. In addition, multiple polymer layers formed by MPC-based polymer coating also displayed similar properties.

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1. Introduction

Adsorption of biomolecules on material surfaces depends on surface properties such as wettability, surface charge, and microstructure [1–3]. Surface modification methods to prevent biofouling, such as polymer coating and grafting, have been investigated for biomedical applications. Polymer brush, owing to its high chain density and well-organized nanostructure, could be a useful surface modification method to generate surfaces with effective anti-biofouling properties [4–7]. Therefore, polymer brush-based surface modification is expected to be applied for advanced biomedical devices.

Polymer brush is synthesized by the surface-initiated polymerization of functional monomer units from substrates. Poly(*N*-isopropylacrylamide) (PIPAAm) brushes have been used for selecting appropriate surfaces for the fabrication of cell sheets [8]. Poly(2-hydroxyethyl methacrylate) (PHEMA) brushes with different graft densities, which show different size-exclusion properties, have been used for the protein separation [9]. Before the practical use of polymer brush-based surface modification is possible, some technical issues need to be solved. One such issue is the immobilization of the initiator to the substrate. The graft density of the polymer brush is highly dependent on the density of the initiator. Currently, because of the lack of initiators, the use of polymer brush in biomedical applications is limited. A successful approach to overcome this challenge involves photo-induced surface-initiated radical polymerization, and this technology has been used for surface modification of artificial joints [10,11]. However, the graft density cannot be precisely controlled in this surface modification; its application in devices that are in contact with blood is therefore risky. In contrast, polymer coating and casting methods are simple and have been used in the biomedical field. For example, a ventricular assist device, EVAHEART, has been successfully coated with the 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer [12,13]. However, the recipients of this implant need lifelong treatment with anti-coagulants such as

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warfarin or aspirin, although MPC-coated pumps showed excellent anti-coagulant properties. Being on an anti-coagulant therapy is inevitable, partly because of the risk of thrombus formation on MPC polymer-coated surfaces [12]. Although coating with the MPC polymer repels plasma proteins, the adsorption of even small amounts of serum proteins could potentially trigger blood coagulation and complement activation. Therefore, surface modification should render the surface completely bio-inert to suppress protein adsorption or cell adhesion, and the polymer brush approach might be useful to achieve this. In the polymer brush approach, high graft density has been reported, and this might be an effective parameter for suppressing protein adsorption [6,7]. Therefore, the polymer chain density is considered an important parameter for anti-protein- and anti-cell-adhesive properties.

In this study, our objective was to identify the most effective design for bio-inert modification by comparing two biocompatible polymers and three different approaches. Here, we used three types of MPC- and ethylene glycol (EG)-based surfaces, polymer brush, monolayer, and polymer-coated surface (Fig. 1); they were selected as they have been reported to be excellent bio-inert surfaces [6,7,14–18]. The structure, particularly chain density, of all the fabricated surfaces was evaluated to investigate the relationship between the structure and anti-protein- and anti-cell-adhesive property.

2. Experimental section

2.1. Materials

MPC was purchased from NOF Co. (Tokyo, Japan). Poly(EG) methyl ether methacrylate (Mn ~300) (mOEGMA), copper (I) bromide (CuBr), 2,2′-bipyridyl (bpy), ethyl-2-bromoisobutyrate, dodecyltrichlorosilane, methanol-d4, bovine serum albumin (BSA), Albumin–fluorescein isothiocyanate conjugate (FITC-BSA) and Pluronic (F-127) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Diisopropylamine and *n*-butyl methacrylate (BMA) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). (3-mercaptopropyl) trimethoxysilane was purchased from Johnson Matthey Japan G.K. (Tochigi, Japan). MPC, mOEGMA, and BMA were used as purchased. Hexane, ethanol, methanol, tetrahydrofuran (THF), acetone, and toluene were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). These solvents were of extra-pure grade and used without further purification. Dulbecco's phosphate buffered saline (PBS, without calcium chloride and magnesium chloride), Dulbecco's modified Eagle's medium (DMEM), and fetal bovine serum (FBS) were purchased from Invitrogen Co. (Carlsbad, CA, USA). L929 (mouse fibroblast) cells were purchased from RIKEN Cell Bank (Ibaraki, Japan).

2.2. Synthesis of (1-(2-butyroiloxyethylphosphorylcholine)propylsulfanyl)trimethoxysilane (MPC-S-Si)

MPC-S-Si was synthesized as described in a previous study [19]. Briefly, MPC (10 mmol), (3-Mercaptopropyl)trimethoxysilane (10 mmol), and diisopropylamine (0.4 mmol) were dissolved in methanol (20 mL). The reaction mixture was bubbled with Ar for 15 min. After 20 h of reaction at 25 °C, methanol was evaporated from the resultant solution (EYELA N-1110, Tokyo Rikakikai Co., Ltd., Tokyo, Japan). The residual solution was washed with hexane and then THF, followed by evaporation again. The obtained product was dissolved in water, freeze-dried, and collected (yield: 1.53 mg [31%]). The obtained product was evaluated by ^{1}H NMR (JNM-GX 270, JEOL, Tokyo, Japan). ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 3.45 (9H, $-CH_3$ of choline group), 0.70 (2H, Si-CH_2-), 3.55 (8.3H, $-OCH_3$ of trimethoxysilane group).

2.3. Synthesis of (1-(2-butyroiloxy(oligoethylene glycol)methyl ether)propylsulfanyl)trimethoxysilane (mOEGMA-S-Si)

mOEGMA-S-Si was synthesized for fabricating the mOEGMA monolayer surface. mOEGMA (10 mmol), (3-Mercaptopropyl)trimethoxysilane (10 mmol), and diisopropylamine (0.4 mmol) were dissolved in methanol (20 mL), and the solution was bubbled with Ar for 15 min. After allowing the reaction to happen for 20 h at 25 °C, methanol was evaporated. The residual mixture was then washed with hexane and THF, evaporated again, and then dried in vacuo (yield: 122 mg [2.5%]). The obtained product was evaluated by ^{1}H NMR. ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 1.25 (3H, $-CH_3$ of methacrylic group), 3.35 (3H, $-CH_3$ of methyl ether group), 0.70 (2H, Si-CH_2-), 3.55 (8.1H, $-OCH_3$ of trimethoxysilane group).

2.4. Fabrication of polymer brush surfaces

(11-(2-Bromo-2-methyl)propionyloxy)undecyltrichlorosilane (BrC10TCS) was synthesized as described in a previous study and used as the initiator for polymer brush fabrication [20]. Si wafers with 10-nm-thick SiO_2 (Furuuchi Chemical Co., Ltd., Tokyo, Japan), SiO_2-coated quartz crystal microbalance with energy dissipation (QCM-D) sensor chips (Q-Sense, Gothenburg, Sweden), and a slide glass (1 cm × 1 cm, Matsunami glass Ind., Ltd., Osaka, Japan) were used as the substrates. These substrates were sonicated in hexane, ethanol, and acetone for 3 min and then subjected to O_2 plasma treatment for 5 min (High voltage, 600 mTorr, PDC-001; Harrick Plasma, Ithaca, NY, USA) for cleaning. The cleaned substrates were kept immersed in 2 mM BrC10TCS solution in toluene at 25 °C overnight. They were then rinsed thoroughly with toluene and dried in vacuo overnight. Polymer brush surfaces of poly(MPC) and poly(mOEGMA) were fabricated on the BrC10TCS-treated substrates by surface-initiated atom transfer radical polymerization (SI-ATRP), according to the protocol reported in a previous study by our group [21]. Briefly, CuBr, 2,2′-bipyridyl (bpy), and MPC or mOEGMA monomer units were dissolved in degassed methanol. The concentrations of CuBr, bpy, and MPC monomer were 0.01 M, 0.02 M, and 0.5 M, respectively. In the case of mOEGMA polymerization, the concentrations of CuBr, bpy, and mOEGMA monomer were 0.03 M, 0.06 M, and 1.5 M, respectively. After the reaction mixture was bubbled with Ar for 10 min at 25 °C, ethyl-2-bromoisobutyrate (the sacrificial initiator; 0.01 M for MPC and 0.03 M for mOEGMA) and the BrC10TCS-treated substrates were immersed in the reaction solution. After 20 h at 25 °C, the reaction was stopped by adding O_2 into the solution. The obtained substrates were sonicated in methanol for 3 min and dried in vacuo overnight. The resultant solution in the fluid phase was collected to examine the reaction ratio by ^{1}H NMR with methanol-d_4 (Reaction ratio >99%).

2.5. Fabrication of MPC and mOEGMA monolayer surfaces

Si wafers with 10-nm-thick SiO_2, QCM sensor chips, and slide glasses were cleaned, as described in the previous section. The substrates were immersed in MPC-S-Si or mOEGMA-S-Si solution (10 mM; in methanol solution bubbled with Ar for 15 min) overnight. The treated substrates were then sonicated with methanol for 3 min and dried in vacuo overnight.

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