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Surface conjugation of zwitterionic polymers to inhibit cell adhesion and protein adsorption

Hsiu-Wen Chien[a,b], Chih-Chi Tsai[a], Wei-Bor Tsai[a,*], Meng-Jiy Wang[c], Wei-Hsuan Kuo[c], Ta-Chin Wei[d], Sheng-Tung Huang[b]

[a] Department of Chemical Engineering, National Taiwan University, No. 1, Sec. 4, Roosevelt Rd., Taipei 106, Taiwan
[b] Graduate Institute of Biochemical and Biomedical Engineering, National Taipei University of Technology, No. 1, Sec. 3, Chung-Hsiao E. Rd., Taipei 106, Taiwan
[c] Department of Chemical Engineering, National Taiwan University of Science and Technology, No. 43, Keelung Rd., Sec. 4, Taipei 106, Taiwan
[d] Department of Chemical Engineering, Chung Yuan Christian University, No. 200, Chung Pei Rd., Chung Li 320, Taiwan

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ABSTRACT

Non-fouling surfaces that resist non-specific protein adsorption and cell adhesion are desired for many biomedical applications such as blood-contact devices and biosensors. Therefore, surface conjugation of anti-fouling molecules has been the focus of many studies. In this study, layer-by-layer polyelectrolyte deposition was applied to create an amine-rich platform for conjugation of zwitterionic polymers. A tri-layer polyelectrolyte (TLP) coating representing poly(ethylene imine) (PEI), poly(acrylic acid)-g-azide and PEI was deposited on various polymeric substrates via layer-by-layer deposition and then crosslinked via UV irradiation. Carboxyl-terminated poly(sulfobetaine methacrylate) p(SBMA) or poly(carboxybetaine methacrylate) p(CBMA) was then conjugated onto TLP coated substrates via a carbodiimide reaction. Our results demonstrate that the zwitterionic polymers could be easily conjugated over a wide pH range except under alkaline conditions, and almost completely block protein adsorption and the attachment of L929 cells and platelets. Therefore, this method has outstanding potential in biomedical applications that require low-fouling surfaces.

1. Introduction

Biomedical devices, upon interaction with biofluids usually elicit non-specific protein adsorption and, in the presence of cells, subsequent cell attachment, which may lead to detrimental clinical complications and poor device performance [1]. Therefore, coatings resisting undesirable protein adsorption and/or cell attachment play a critical role in many biomedical applications, such as diagnostic assays, biosensors and blood-contacting devices. The most commonly employed strategy for preparing anti-fouling surfaces is the surface conjugation of oligo- or poly(ethylene glycol) (PEG) [2–6]. Due to its highly hydrophilic nature, PEG exhibits considerable flexibility and high mobility in aqueous solution [7]. The mechanism for the protein resistance of PEG has been explained by the high mobility of the polymer, the large excluded volume and steric hindrance effects [8,9].

Zwitterionic materials are overall neutral molecules containing positively and negatively charged groups, such as phosphobetaine, sulfobetaine, and carboxybetaine, have been used as anti-fouling materials. Formation of a highly hydrated surface due to the high water-binding capacity of zwitterions is considered to be the key for its anti-fouling properties. Furthermore, charge neutrality of zwitterionic molecules is another key factor for reducing interactions with proteins and cells. Phosphorylcholine, a phosphobetaine, appearing in phospholipids of the outer layer of cell membranes, was first fabricated as the methacrylate monomer, 2-methacryloyloxyethyl phosphorylcholine (MPC). Surface polymerization of MPC enhances protein resistance of surfaces and improves blood compatibility [10,11]. However, MPC is difficult to synthesize [12]. On the other hand, two other zwitterionic monomers, sulfobetaine methacrylate (SBMA) and carboxybetaine methacrylate (CBMA), were relatively simple to synthesize. Their polymers also show excellent antifouling ability to prevent protein adsorption, platelet adhesion and cell adhesion [13–19].

Many studies have also focused on the conjugation of zwitterionic polymers on biomaterials to create a non-fouling surface. For example, a block copolymer of SBMA and hydrophobic poly(propylene oxide) was physically adsorbed on hydrophobic substrates and prevented fibrinogen adsorption [20]. Alternatively, copolymers of SBMA and negatively charged acrylic acid could adsorb on a positively charged surface to prevent platelet adhesion [17]. Nevertheless, covalent grafting of p(SBMA) is more desirable

* Corresponding author. Tel.: +886 2 3366 3996; fax: +886 2 2362 3040.
E-mail address: weibortsai@ntu.edu.tw (W.-B. Tsai).

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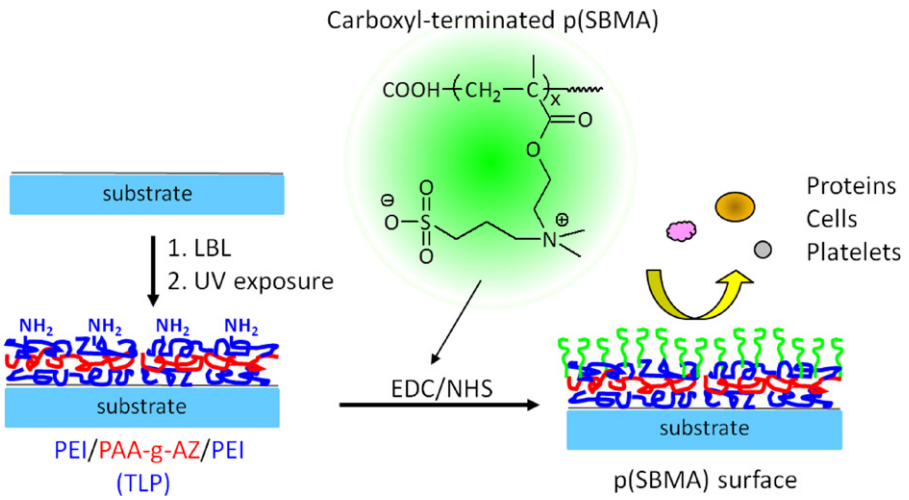

Scheme 1. Illustration of surface conjugation of p(SBMA).

compared with physical adsorption for surface stability. One of the strategies for surface grafting is the "graft-from-surface" method, i.e., polymerization of SBMA from surface-conjugated initiators. On the other hand, the "graft-to-surface" method conjugates preformed polymers onto a surface. To this end, zwitterionic polymers need to possess a reactive group that can form covalent bonds with the substrate. For example, p(SBMA) or p(CBMA) containing catechol residues was grafted onto Au and glass surfaces to resist protein adsorption and cell adhesion [21,22].

The "graft-to-surface" method also needs reactive groups on the surface to form covalent bonds with end-functional groups of zwitterionic polymers. For a chemically inert surface, surface functionalization is therefore needed prior to surface grafting. In this study, we applied a simple surface modification technique, layer-by-layer (LBL) polyelectrolyte deposition, to create an amine-rich surface for conjugation of p(SBMA) (Scheme 1). This technique is based on the alternate adsorption of positively and negatively charged polyelectrolytes to build an ultra-thin film onto a substratum. We previously deposited a tri-layer polyelectrolyte (TLP) film of poly(ethylene imine) (PEI), poly(acrylic acid)-g-azide (PAA-g-AZ) and PEI on substrates. After exposure to UV irradiation, the TLP film is crosslinked via a phenylazide reaction and covalently conjugated on the substrates [17]. Since PEI is on the topmost layer, amino groups are exposed on the surface. In this study, poly(SBMA) with a carboxylated end was synthesized with varied chain length, and then was conjugated onto TLP coated substrates. The efficacy in inhibiting cell adhesion and protein adsorption was then evaluated. Furthermore, poly(CBMA) was conjugated on TLP coated substrates via the same strategy to prevent cell adhesion.

2. Materials and methods

2.1. Materials

Sulfobetaine methacrylate (SBMA; 2-methacryloyloxy ethyl dimethyl-3-sulfopropyl, cat #537284), poly(acrylic acid) (PAA, cat #523925) and poly(ethyleneimine) (PEI, Mw. ~ 750 kDa, cat #P3143) were received from Sigma–Aldrich (St. Louis, USA). Poly(acrylic acid-g-azidoaniline) (PAA-g-AZ) was synthesized by conjugating 4-azidoaniline on PAA via a carbodiimide reaction, according to a previously published protocol [23]. Sheep anti-human fibrinogen antibody (cat #AHP061) was received from Serotec, while horse radish peroxidase (HRP)-conjugated anti-sheep IgG antibody (cat #sc-2770) were provided by Cruz Biotechnology. Rabbit anti-fibronectin antibody (cat #F3648) and HRP-conjugated anti-rabbit IgG antibody (cat #A6154) were purchased from Sigma–Aldrich. N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) was purchased from Fluka (USA). All other chemicals were purchased from Sigma–Aldrich unless specified otherwise.

L929 mouse fibroblast-like cells were received from Food Industry Research and Development Institute (Hsinchu, Taiwan). L929 cell culture medium contained alpha minimum essential medium (αMEM; HyClone, USA) supplemented with 10% fetal bovine serum (JRH, Australia), 2 mg/mL $NaHCO_3$, 0.5% of fungizone (GIBCO), 0.25% gentamycin (GIBCO) and 0.679% β-mercaptoethanol. Platelet suspension buffer was prepared by 137 mM NaCl, 2.7 mM KCl, 5.5 mM glucose, 0.35% (w/v) bovine serum albumin (BSA), 3 mM Na_2HPO_4, 3.5 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 0.1 U/mL apyrase, pH 7.4. Phosphate-buffered saline (PBS) was prepared with 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.8 mM KH_2PO_4 at pH 7.4.

2.2. Polymer synthesis

Carboxyl-terminated p(SBMA) was synthesized by free radical polymerization using 4,4′-azobis(4-cyanovaleric acid) as the initiator. Briefly, 0.5 g SBMA was dissolved in 15 mL deionized water and preheated to 60 °C. The initiator was dissolved in 1 mL methanol, and then added to the SBMA solution with the molar ratios of SBMA/initiator were 7.5 or 20. The two types of p(SBMA) were abbreviated as $p(SBMA)_{7.5}$ and $p(SBMA)_{20}$. The reaction was underwent at 60 °C for 24 h and terminated in an ice bath. The unreacted monomers and small oligamers were removed by dialysis against deionized water (dialysis tubing cellulose membrane, MWCO 3.5 kDa), and the polymers were freeze-dried. The carboxyl-terminated p(SBMA) was investigated by using a Fourier transform infrared microscopy (Fig. S1 in the supplementary). The adsorption bands at 1748 cm^{-1} (C═O), 1454 cm^{-1} ($CH_2C(=O)O$), and 1240 and 1175 cm^{-1} (C—O—C) are characteristic of ester group of SBMA, while a peak at 3552 cm^{-1} (O—H) is attributed to the characteristic band of carboxylic acid groups. Although we suggest that the molecular weight of $p(SBMA)_{20}$ is higher than that of $p(SBMA)_{7.5}$, we had difficulty in determining the molecular weight of $p(SBMA)_{7.5}$ and $p(SBMA)_{20}$ by gel permeation chromatography, since p(SBMA) tends to aggregate.

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.colsurfb.2013.01.071.

2-Carboxy-*N*,*N*,-dimethyl-*N*-(2′-(methacryloyloxy)ethyl) ethanaminium inner salt (carboxybetaine methacrylate, CBMA)