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Functionalizable and ultra-low fouling zwitterionic surfaces via adhesive mussel mimetic linkages

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ABSTRACT

In this work, a biomimetic polymer (pCB_2-$catechol_2$), with two zwitterionic poly(carboxybetaine) (pCB) arms for ultra-low fouling and two adhesive catechol groups for surface anchoring, was developed. Two pCB arms were grown from an initiator with two catechol groups via atom transfer radical polymerization (ATRP). Binding tests of pCB_2-$catechol_2$ were performed on a gold surface under a range of conditions such as pH values and solvents. Protein adsorption from single protein solutions of fibrinogen and lysozyme, and complex media of 100% blood plasma and serum was evaluated using a surface plasmon resonance (SPR) sensor. Results are compared with those from two other polymers (i.e., one polymer with one pCB chain and one catechol group, termed as pCB-catechol, and another polymer with one pCB chain and two catechol groups, termed as pCB-$catechol_2$). Furthermore, the direct immobilization of anti-activated leukocyte cell adhesion molecule (anti-ALCAM) was carried out on the pCB_2-$catechol_2$ modified surface. Results showed that the antibody-immobilized surface maintained its excellent ultra-low fouling properties. The detection of activated leukocyte cell adhesion molecule (ALCAM) in 100% blood plasma with high sensitivity and specificity was achieved. This work demonstrates an effective and convenient strategy to obtain functionalizable and ultra-low fouling surfaces.

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1. Introduction

Surfaces resisting non-specific adsorption have played a very important role for diagnostics, drug delivery and implantable devices. Several materials, such as poly(ethylene glycol) (PEG) or oligo ethylene glycol (OEG) [1,2], tetraglyme [3], dextran [4], mannitol [5], polyamines functionalized with acetyl chloride [6], PEG-mimetic peptoid [7], serine-rich peptides [8], and zwitterionic materials [9–15] have been developed for this purpose. While PEG or OEG has been widely used, there is only one functionalizable group at the end of each chain [16,17]. For early cancer diagnostics, it is highly desirable to have a surface platform, which is highly specific and sensitive due to low concentration of biomarkers available in complex media [18]. Our recent results showed that zwitterionic polymers, such as poly(carboxybetaine)(pCB) [13,18,19], not only highly resist non-specific adsorption from complex media, but also have abundant functional groups for the convenient immobilization of molecular recognition elements.

Previously, zwitterionic polymers were usually coated onto surfaces by surface-initiated ATRP, a "graft-from-surface" method, with excellent ultra-low fouling properties. However, ATRP requires surface grafted initiators/catalysts and oxygen-free conditions. For practical applications, it is desirable to use a simpler and more convenient method to attach zwitterionic polymers onto a surface, for example, by simply treating the surface with a polymer solution using a "graft-to-surface" method. Polymers used in the "graft to" method, usually compose of adhesive moieties, enabling the adhesion of non-fouling polymers onto the surface. Messersmith et al. [20] reported that dopamine could be coated on a wide range of inorganic and organic materials, including noble metals, oxides, polymers, semiconductors, and ceramics. Dopamine with catechol and primary amine groups can self-polymerize to form thin, surface-adherent polydopamine films at pH ~ 8.5 [20]. Polymers (e.g., PEG and PEG-mimetic peptoid) with adhesive 3,4-dihydroxyphenyl-L-alanine (DOPA) groups were used to modify TiO_2 surfaces at pH 6.0–7.4 [21,22]. These polymers appear to be less effective for immobilization onto other surfaces (e.g., Au and SiO_2) [23,24]. Zurcher et al. [25] reported a new DOPA-containing structure based on iron chelator anachelin for the anchoring of PEG onto TiO_2 surface under optimal conditions (50 °C, 1.2 M ionic strength, K_2SO_4/NaCl 1:1). Recently, Li et al. showed that poly(sulfobetaine methacrylate) with a catechol residue (pSBMA-

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catechol) grafted onto NH_2-modified Au and glass surfaces was highly resistant to protein adsorption and biofilm formation [26]. However, pSBMA-catechol is very hard to be functionalized.

The objective of this work is to develop a zwitterionic pCB with biomemic adhesive catechol groups so as to create a functionalizable surface in ultra-low fouling zwitterionic background via the convenient "graft to" approach. With a newly developed initiator, a polymer with two adhesive catechol groups for anchoring and two pCB chains for creating high polymer packing densities on a surface can be synthesized via ATRP. The catecholic oxygens need to be protected in order to polymerize CB monomers via ATRP since catechol will act as a radical inhibitor during polymerization. This surface platform was then used to demonstrate the detection of cancer biomarkers using a surface plasmon resonance (SPR) sensor. For early cancer diagnosis, the difference in cancer biomarkers between cancer patients and healthy persons can often be quite small. For example, the normal level of activated leukocyte cell adhesion molecule (ALCAM, CD 166), a potential marker of various carcinomas, is ~84 ng/mL for healthy people. However, above 100 ng/mL ALCAM in blood indicates that the patient potentially may have cancer(s) [27]. Existence of these biomarkers in complex blood media offers another challenge in diagnostics. This new platform allows one to functionalize anti-ALCAM onto ultra-low fouling pCB background easily and to make it possible to detect ALCAM down to ~30 ng/mL in 100% human blood plasma.

2. Materials and methods

2.1. Materials

3,4-Dihydroxyphenyl-L-alanine (DOPA), 2-bromoisobutyric acid, dicyclohexyl carbodiimide (DCC), *N*-hydrosuccinylimide, copper(I) bromide (99.999%), 2,2′-bipyridine (BPY 99%), Fg (fraction I from bovine plasma), Lyz (from chicken egg white), phosphate buffer saline (PBS, pH 7.4, 0.15 M, 138 mM NaCl, 2.7 mM KCl), and tetrahydrofuran (THF HPLC grade) were purchased from Sigma–Aldrich. Tetrabutylammonium fluoride (TBAF, 1 M solution in THF containing ca 5% water), 1,3-diamino-2-hydropropane, and tert-butyl chlorodimethylsilane (TBDMS, 98%) were purchased from Acros. Pooled human plasma and serum were purchased from BioChemed Services (Winchester, VA). Water used was purified using a Millipore water purification system with a minimum resistivity of 18.0 MΩ cm. *N*,*N*-dimethylformamide and dichloromethane were dried with phosphorous oxide and then distilled. *N*-succinimidyl 2-bromoisobutyrate (**1**) [28], 3,4-bis(terbutyldimethylsilyoxyl)-L-phenylalanine (**2**) [29], Boc-($DOPA_2$(TBDMS)$_4$-NHS) [21], CBMA monomer [13], and trifluoroacetic acid salt of 2-aminoethyl 2-bromoisobutyrate [30] were prepared following the procedures reported previously. Molecular weights of the polymers were determined using an aqueous gel permeation chromatograph (GPC) (Waters 2695 Separations Module) with a Waters 2414 refractive index detector and a Waters ultrahydrogel 250 column (7.8 mm × 300 mm). The buffer solution (0.05 M Tris buffer + 1.0 M NaCl) was used as the eluent with a flow rate of 0.5 mL/min at 35 °C. All samples were filtered through 0.2 μm PTFE filters prior to injection. The system was calibrated with narrow molecular weight polyethylene oxide standards.

2.2. 2-Bromo-2-methyl-N-1-carboxyl-2-[3,4-bis(t-butyldimethylsilyloxy)]-phenyl-ethyl propionamide (**3**)

N-Succinimidyl 2-bromoisobutyrate (0.528 g, 2.00 mM) was dissolved in dry DMF (2.5 mL) and 3,4-bis(terbutyldimethylsiloxyl)-L-phenylalanine(0.85 g, 2.00 mM) was added at once under N_2. The mixture was stirred on an ice-bath, then diisopropylethylamine (DIEA) (350 μL, 2 mM) was added via a syringe. The reaction mixture was kept in ice-bath for one hour. Its temperature was raised to room temperature for overnight. The mixture was treated with diluted solution of HCl (5%, 40 mL) and then extracted with EtOAc (30 mL). The organic layers were combined and washed with DI water (30 mL), and dried with anhydrous $MgSO_4$. The crude product was purified with silica gel with chloroform and 1% methanol as an eluent. 3,4-Bis(*t*-butyldimethylsilyloxy)-*N*-isobutyryl-L-phenylalanine was obtained as a white foam, (1.09 g, 90%). ^{1}H NMR($CDCl_3$), δ: 7.01–7.04 (d, 1H), 6.63–6.80 (m, 3H), 4.73–4.75 (m, 1H), 3.05–3.15 (m, 2H), 1.88–1.92 (d, 6H), 0.99(s, 18H), 0.20 (s, 12H).

2.3. 2-Bromo-2-methyl-N-1-(carbonyloxysuccinimide)-2-[3,4-bis-(t-butyldimethylsilyloxy)]phenyl-ethyl propionamide (**4**)

Compound (**4**) was synthesized using the same synthetic procedure as **1**, and the yield is 85%. ^{1}H NMR($CDCl_3$), δ: 7.01–7.03 (d, 1H), 6.64–6.79 (m, 3H), 4.72–4.74 (m, 1H), 3.05–3.15(m, 2H), 2.86 (s, 4H), 1.88–1.92 (d, 6H), 0.99 (s, 18H), 0.20 (s, 12H).

2.4. N,N′-(2-Hydroxy-1,3-propanediyl)-bis[2-(N-2-bromo-2-methyl propionamide)–3-(3,4-di(t-butyldimethylsilyloxy))phenyl]propanamide ($Catechol_2$-Br_2) (**5**)

The initiator **5** was synthesized from **4** and 1,3-diamino isopropyl alcohol. Briefly, **4** (1.4 g, 2.08 mmol) was dissolved in dry DMF (6 mL) and 1,3-diamino isopropyl alcohol (90 mg, 1.00 mmol) was added at once under N_2. The mixture was stirred on an ice-bath, then diisopropylethylamine (DIEA) (385 μL, 2.2 mM) was added via a syringe. The reaction mixture was kept in ice-bath for one hour. Its temperature was raised to room temperature for overnight. The mixture was treated with diluted solution of HCl (5%, 40 mL) and then extracted with EtOAc (30 mL). The organic layers were combined and washed with 30 mL DI water, dried ($MgSO_4$) and evaporated. The crude product was loaded onto a silica gel column with chloroform and 1% methanol as the eluent. **5** was obtained as a yellow powder, the yield was 0.78 (65%). ^{1}H NMR ($CDCl_3$), δ: 7.06 (m, 2H), 6.88 (m, 2H), 6.65–6.79 (m, 6H), 4.46–4.51(m, 2H), 3.75–3.78(t, 1), 2.90–3.46 (m, 8H), 1.84–1.93 (d, 12H), 0.99 (s, 36H), 0.19 (s, 24H).

2.5. Preparation of pCB_2-$catechol_2$ (**6**)

Initiator **5**, 26 mg (0.022 mM), BPY (20 mg, 0.13 mM), CuBr, 6.80 mg (0.047 mM), and $CuBr_2$, and 1.03 mg (0.005 mM) were placed into a three-necked flask. The system was degassed three times and filled with N_2, then 1 mL DMF (degassed) was added under N_2. The mixture was stirred for 20 min at room temperature. 1.0 g CBMA, dissolved in H_2O/DMF (2 mL/7 mL) was added into the reaction system. The polymerization reaction continued for 10 h. The resulting polymer precipitate was collected by filtration, and dissolved in H_2O again. The polymer solution was dialyzed for 2 days with DI water. The white powder (0.9 g, 90%) was obtained after removal of water. Before surface adhesion, the TBDMS groups of protected pCB_2-$catechol_2$ were removed using TBAF in order to achieve complete deprotection. A solution with 1.0 mM TBDMS protected pCB_2-$catechol_2$ and 10 mM TBAF in THF was stirred overnight. The suspension was centrifuged and the supernatant was removed. The remaining white powder (**6**) was washed three times with THF and dried under reduced pressure.

2.6. Preparation of 2-Boc-$DOPA_2$-$(TBDMS)_4$-amidoethyl 2-bromoisobutyrate ($Catechol_2$-Br) (**7**)

Boc-$DOPA_2$(TBDMS)$_4$-NHS [21] (1032 mg, 1.00 mM) was dissolved in dry DMF (5 mL) and trifluoroacetic acid salt of 2-aminoethyl 2-bromoisobutyrate [30] (339 mg, 1.00 mM) was added at once under N_2. The mixture was stirred on an ice-bath, then diisopropylethylamine (DIEA) (385 μL, 2.2 mM) was added via a syringe. The reaction mixture was kept in ice-bath for one hour. Its temperature was raised to room temperature for overnight. The mixture was treated with diluted solution of HCl (5%, 40 mL) and then extracted with EtOAc (30 mL). Organic layers were combined and washed with 30 mL DI water, dried ($MgSO_4$) and evaporated. The crude product was loaded onto a silica gel column with chloroform and 1% methanol as the eluent. **7** was obtained as a white foam, (1.03 g, 91%). ^{1}H NMR($CDCl_3$) δ: 6.60–6.82(m, 6H), 6.38–6.44(m, 2H), 4.64–4.67(m, 2H), 4.12–4.19(m, 2H), 4.09–4.11(m, 1H), 3.14–3.60 (m, 3H), 2.66–3.04(m, 3H), 1.95(d, 6H), 1.31(s, 9H), 1.0 (m, 36H) 0.2 (m, 24H).

2.7. Preparation of pCB-$catechol_2$ (**8**)

Initiator 7, 52 mg (0.05 mmol), BPY (44 mg, 0.29 mmol), CuBr, 13.6 mg (0.094 mmol), and $CuBr_2$, 1.03 mg (0.005 mmol), were placed into a three-necked flask. The system was degassed three times and filled with N_2, then 1 mL DMF (degassed) added under N_2. The mixture was stirred for 20 min at room temperature. 1.0 g CBMA, dissolved in H_2O/DMF (2 mL/7 mL) was added into the reaction system. The polymerization continued for 10 h. The resulting polymer precipitate was collected by filtration, and dissolved in H_2O again. The polymer solution was dialyzed for 2 days with DI water, and white polymer powder (0.85 g, 85%) was obtained after removal of water. The molecular weight and molecular weight distribution of protected p-CB-$catechol_2$ were measured with GPC, Mn: 80 800 (against PEO), PDI is 1.22. The resulting polymer was deprotected with the same procedure as polymer 6 before using.

2.8. Preparation of pCB-catechol

pCB-catechol was synthesized following the method reported before for pSBMA-catechol [26].

2.9. Grafting pCB_2-$catechol_2$ onto bare Au chip

10 mg deprotected polymer was dissolved in 2 mL DI water (pH = 3) in a 20 mL glass tube. 1 mL THF was added dropwise into this tube. The turbid polymer solution was sonicated for 10 min, then transferred to a Teflon cell. The cleaned Au chip, prepared as described in [20], was placed into the cell and submerged for 24 h. The chip coated with pCB_2-$catechol_2$ by this method was washed with DI water twice and dried with airflow before loaded on the SPR instrument.