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Amino Acid-Based Zwitterionic Poly(serine methacrylate) as an Antifouling Material

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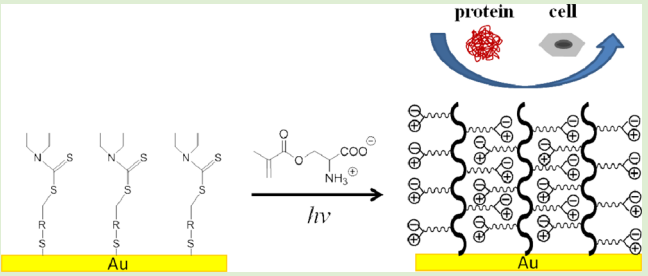
ABSTRACT: A serine-based zwitterionic poly(serine methacrylate) (pSerMA) was developed in this work to be used as a potential antifouling material. A surface-initiated photoiniferter-mediated polymerization (SI-PIMP) method was used to graft polymer brushes on gold surfaces. The pSerMA-grafted samples with different polymer film thicknesses were readily prepared by varying the UV-irradiation time. With the optimal film thickness, the adsorptions from bovine serum albumin, human serum, and human plasma onto the pSerMA-grafted surfaces, as evaluated by a surface plasmon resonance (SPR) biosensor, were 1.8, 9.2, and 12.9 ng/cm^2, respectively, comparable to the traditional antifouling material such as poly(ethylene glycol). The pSerMA-grafted surfaces also strongly resisted adhesion from bovine aortic endothelial cells. This is the first work to develop an amino acid-based zwitterionic polymer as an antifouling material, demonstrating that pSerMA is a promising alternative to the traditional ethylene glycol-based antifouling materials.



INTRODUCTION

Surfaces with strong fouling resistance are of critical importance in many applications, such as biocompatible and functional biomedical implants,[1] biosensors,[2] drug delivery,[3,4] and ship hulls.[5] Fouling surfaces may cause deleterious biological processes, for instance, platelet adhesion and aggregation and foreign body reaction, potentially leading to device failure.[6−8] Therefore, it is necessary to develop materials capable of reducing or eliminating adsorption of protein, especially in the complex media such as blood plasma and serum.

Ethylene glycol-based materials such as poly(ethylene glycol) (PEG) and oligo(ethylene glycol) (OEG) are currently the most prevalent antifouling materials.[9−11] A major limitation of the ethylene glycol-based materials is their susceptibility to oxidative degradation.[12,13] Other antifouling materials reported in recent years include hydrophilic polymers such as polysaccharides,[14] polypeptoids,[15,16] poly(acrylamide),[17] and poly(hydroxypropyl methacrylate)[18] and zwitterionic-based materials such as poly(sulfobetaine methacrylate) (pSBMA),[19,20] poly(carboxybetaine methacrylate) (pCBMA),[21] poly(carboxybetaine acrylamide) (pCBAA),[22] and poly(2-methacryloyloxyethyl phosphorylcholine).[23] Among them, zwitterionic materials, carrying zwitterions of carboxybetaine or sulfobetaine, have drawn the most attention due to their ultralow biofouling properties, which are attributed to their strong hydration capacity dictated by electrostatic interactions between zwitterions and water.[24]

Amino acids are natural zwitterions, composed of an asymmetric α carbon at their center, an amine group ($-NH_2$), a carboxyl group ($-COOH$), a hydrogen atom, and a side chain specific to each amino acid. The unique zwitterionic and biomimetic nature of amino acids has prompted us to design new zwitterionic antifouling polymers incorporating amino acids.

A few previous works explored the zwitterionic structure-responsible properties of the amino acid-containing materials, in applications such as biofouling suppression, removal of heavy metal ions, and ionic transport in nanochannels.[25−31] Shiraishi reported that the microspheres of copolymer of zwitterionic *O*-methacryloyl L-serine (SerMA) and methyl methacrylate (MMA) had reduced adsorption of albumin and fibrinogen, as compared to the PMMA microspheres.[25] A low-fouling zwitterionic surface was realized by functionalizing silica nanoparticles with cysteine via its thiol group, which displayed enhanced stability (i.e., less particle aggregation) in solutions of lysozyme, bovine serum albumin (BSA), and diluted human serum, as compared to the unmodified particles.[26] In another study, short-chain lysine was grafted onto the surface of the polyacrylonitirile porous filtration membrane; the modified membrane showed reduced BSA and lysozyme adsorption.[27] Romanski et al. incorporated the acryloyl and methacryloyl lysine into polymeric hydrogels for the sorption and release of heavy metal ions.[28] Phosphatidylserine was found to bind copper ions with high affinity in a reversible and pH-dependent fashion.[29] In Azzaroni's work, zwitterionic poly(methacryloyl-L-lysine) brushes was utilized to modify nanopore surfaces to modulate the ionic transport.[30,31]

So far, the work on antifouling performance of the amino acid-based zwitterionic materials has been very limited, using either single amino acids or copolymers containing amino acid moieties.[25−27] The antifouling properties and full potential of

Received: October 25, 2012
Revised: December 8, 2012
Published: December 10, 2012

dx.doi.org/10.1021/bm301646y | *Biomacromolecules* 2013, 14, 226−231

the amino acid-based zwitterionic polymers have been barely studied. Also there has been no quantitative study on the resistance of such materials to protein adsorption from complex media such as serum or plasma.

In this work, we developed a serine-based zwitterionic poly(serine methacrylate) (pSerMA) and explored its antifouling properties. The polymer was grafted on gold surfaces via a surface-initiated photoiniferter-mediated polymerization (SI-PIMP) method. Protein adsorption from BSA, full serum, and full plasma were investigated by a surface plasmon resonance (SPR) biosensor. Resistance of the pSerMA-grafted surfaces to cell adhesion was also studied. To the best of our knowledge, this is the first work to develop the amino acid-based zwitterionic homopolymer as an antifouling material and to graft the gold surface with pSerMA brushes to achieve an antifouling surface.

MATERIALS AND METHODS

Materials. 11-mercapto-1-undecanol (97%), 4-(chloromethyl)-phenyl isocyanate (97%), dibutyltin dilaurate (95%), iodine, and copper(II) carbonate basic were purchased from Sigma-Aldrich (Milwaukee, WI). Diethylammonium diethyldithiocarbamate (98%), tributylphosphine (95%), methacryloyl chloride (97%), L-serine (99%), and 8-hydroxyquinoline were obtained from Alfa Aesar (Ward Hill, MA). The absolute 200 proof ethanol was purchased from PHARMCO-AAPER. Chloroform (99.5%), tetrahydrofuran (THF, 99%), acetone (99.5%), methanol (99.8%), and ether (99%) were all obtained from Sigma-Aldrich.

BSA and phosphate-buffered saline (PBS, pH 7.4, 10 mM, 138 mM NaCl, 2.7 mM KCl) were purchased from Sigma-Aldrich. Pooled human blood plasma and serum were purchased from BioChemed Services (Winchester, VA). The plasma was anticoagulated with citrate-phosphate-dextrose. Water used in all experiments was purified using a Millipore system to reach a resistivity above 18.0 MΩ·cm. Bovine aortic endothelial cells (BAECs) were supplied by Prof. Shaoyi Jiang at the University of Washington. Dulbecco's modified Eagle medium (DMEM, with 4.5 g/L glucose, 4.0 mM L-glutamine, and 110 mg/L sodium pyruvate) was acquired from Thermo Scientific (Waltham, MA). All other cell culture reagents were purchased from Invitrogen (Grand Island, NY).

Synthesis of the SerMA Monomer. The SerMA monomer was synthesized using a method similar to one reported previously.[32] L-serine (25 g, 238 mmol) was dissolved in 250 mL water at 90 °C. Basic cupric carbonate (29 g, 131 mmol) was then added into the solution and stirred for 10 min. After the insoluble residue was filtered, 50 mL acetone was put into the solution, followed by the addition of 135.5 mL of 2 M KOH aqueous solution. Methacryloyl chloride (28.8 mL, 297.5 mmol), diluted in 50 mL acetone, was then added dropwise at 4 °C over 20 min. The reaction was carried out overnight at room temperature under stirring conditions. The blue precipitate of the SerMA copper complex was then filtrated and washed successively with water, methanol, and ether.

Next, powder of the SerMA copper complex (22.8 g, 55.8 mmol) was added into a solution of 8-quinolinol (9.70 g, 66.9 mmol) in chloroform (300 mL). Three hundred milliliters of water were then added. After shaking overnight, green precipitates in the chloroform layer, 8-quinolinol copper complex, were removed by filtration. The water phase was concentrated to 50 mL and the product in water was recrystallized from THF to yield SerMA as white powder. ^{1}H NMR (D_2O, 300 MHz): δ = 1.85 (s; 3H, CH_3), 4.06 (t, 1H, CH), 4.53 (d, 2H, CH_2), 5.68 (s, 1H, C═CH_2), 6.09 (s, 1H, C═CH_2).

SI-PIMP. The photoiniferter 11-mercaptorundecane-1-[4-({[(diethylamino)-carbonothioyl] thioethyl}phenyl)carbamate] (DTCA) was synthesized following the procedure reported previously.[33,34] The structure of the photoiniferter was confirmed by ^{1}H NMR.

The SPR chip was prepared by depositing an adhesion-promoting chromium layer (2 nm) and a surface plasmon-active gold layer (48 nm) onto the glass substrate by e-beam evaporation under vacuum. Prior to the preparation of the self-assembled monolayer (SAM) of the photoiniferter DTCA on gold, the SPR chip was rinsed by acetone, ethanol, and water, treated under UV/ozone for 20 min, washed by water and ethanol, and dried. The photoiniferter SAM was formed by soaking the cleaned chip in 1 mM photoiniferter in THF overnight at room temperature. The chip was then rinsed with THF and dried with a stream of filtered air.

The modified chip was put into a quartz tube and protected under N_2. Ten milliliters of 34.6 mg/mL SerMA monomer solution in PBS was purged with N_2 for 30 min and transferred to the quartz reaction tube using a syringe. Samples were then irradiated with a 302 nm UV lamp (UVP, model UVM-57) coupled with a 280 nm cutoff filter for the desired reaction time. The cutoff filter was used to avoid the cleavage of the thiol-gold bond of the photoiniferter SAM.[34] After reaction, the chips were washed with water and PBS and kept in PBS before use.

Ellipsometry. The pSerMA-grafted chips were washed with water and dried with an air flow before the ellipsometry measurements. The film thickness of the polymer layer on gold was evaluated by an α-SE ellipsometer (J. A. Woollam Co., Lincoln, NE) equipped with a 632.8 nm He−Ne laser at incidence angles of 65−75°. A refractive index of 1.45 was assigned to the polymer layer.

Contact Angle Measurements. Static contact angles of water on the photoiniferter SAM or pSerMA-grafted gold surfaces were measured by the sessile drop technique under ambient conditions, using a Rame-Hart goniometer (model 100−00, Mountain Lakes, NJ). At least three readings from different parts of the film were taken and averaged.

Protein Adsorption Measurements by SPR. Protein adsorption was evaluated with a four-channel SPR sensor (PLASMON-IV, Institute of Photonics and Electronics, Academy of Sciences, Czech Republic). The sensor measures change in the resonant wavelength at a fixed light incident angle.

The pSerMA-grafted SPR chip was first attached to the base of the prism. Optical contact between two surfaces was realized using a refractive index matching fluid (Cargille). A preadsorptive baseline was first established by flowing PBS buffer over the chip surface for 10 min. The BSA solution (1 mg/mL), 100% blood serum, or 100% blood plasma was then run through different flow channels for 10 min, followed by flushing the chip surface with PBS for 5 min to establish the postadsorptive baseline. A flow rate of 0.05 mL/min was used for all experiments. The SPR chips without any modification or modified only with the photoiniferter SAMs were used as control for comparison. Protein adsorption was finally quantified by measuring wavelength shift between the preadsorptive and postadsorptive baselines and converting it to the amount of the adsorbed protein. A 1-nm SPR wavelength shift at 750 nm, for the sensor used in this work, corresponds to a protein surface coverage of 15 ng/cm^2.[16]

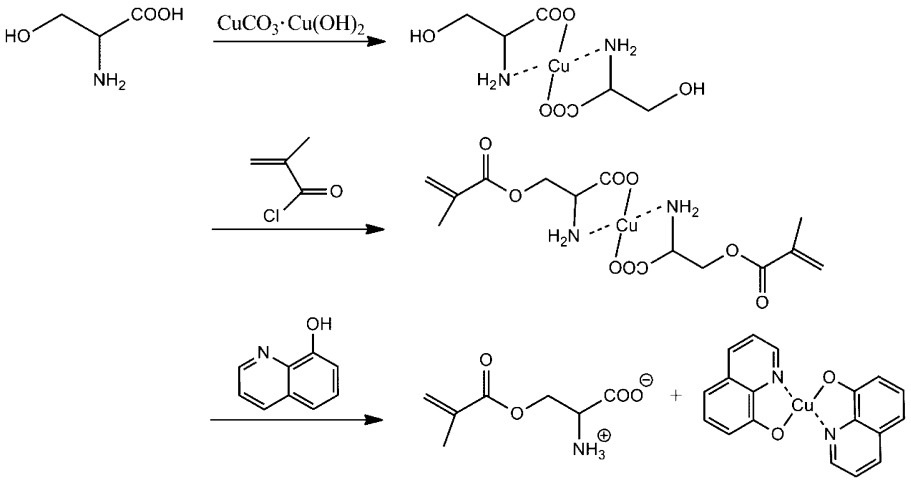
Cell Adhesion. BAECs were maintained in continuous growth in a humidified incubator with 5% CO_2 at 37 °C. The culture medium was composed of DMEM, 10% fetal bovine serum (FBS), 1% sodium pyruvate, 1% nonessential amino acids, and 2% penicillin streptomycin. Cells were passaged once a week and used only before passage 15.

The pSerMA-grafted or bare gold-coated glass substrates were transferred to individual wells of a 24-well plate and rinsed with sterile PBS three times. Cells were harvested by treatment with trypsin/ethylenediaminetetraacetic acid (0.05%/0.53 mM) to detach the adherent cells, washed with PBS, and subsequently diluted in the culture medium to reach a final concentration of 10^5 cells/mL. Two milliliters of cell suspension were then added into each well and incubated with the samples for 7 days, with culture medium refreshed every 3 days. Phase-contrast images (10 ×) were finally acquired using an EVOS xl core inverted microscope (Advanced Microscopy Group, Bothell, WA).

RESULTS AND DISCUSSION

SerMA, the monomer derived from a natural α-amino acid (L-serine), can exist as a zwitterion in aqueous solutions. With

Scheme 1. Synthesis of the SerMA Monomer



rational designs, the zwitterionic SerMA might be developed into a promising candidate to resist fouling for proteins and cells.

The SerMA monomer was first synthesized via the reaction of methacryloyl chloride and L-serine, according to Scheme 1. Structure of the monomer was confirmed by NMR (Figure 1).

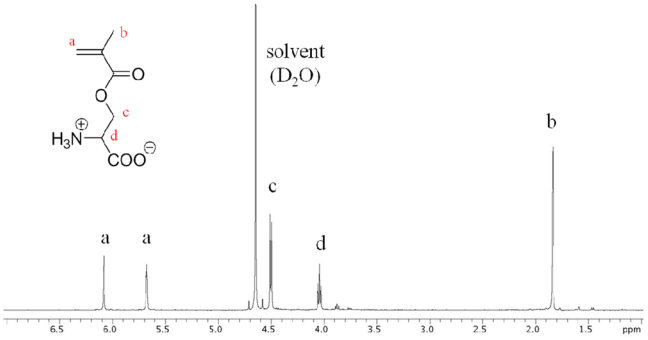
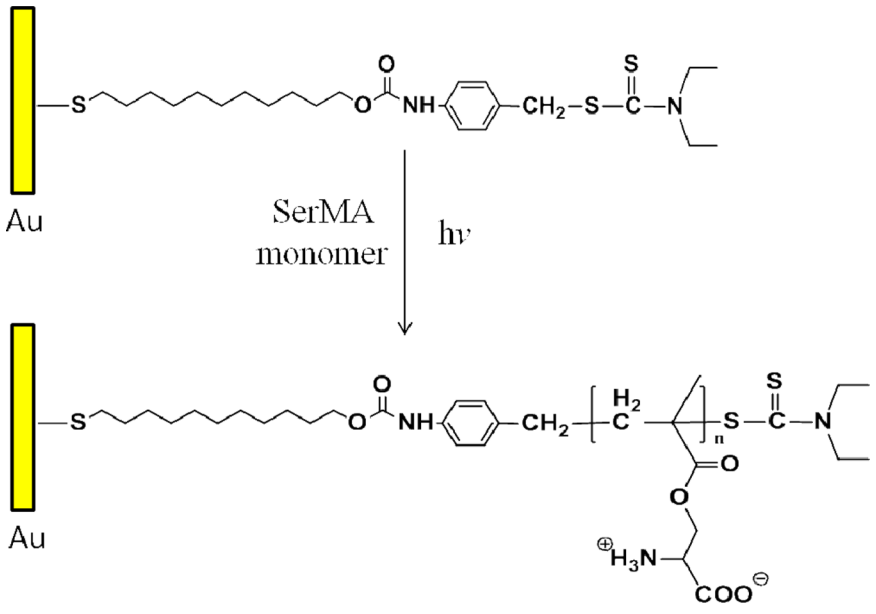


Figure 1. ^{1}H NMR spectrum of the SerMA monomer.

The poly(*O*-methacryloyl L-serine) (poly(serine methacrylate), pSerMA) brushes were then prepared on gold surfaces following a 2-step procedure (Scheme 2). First, the photoiniferter SAMs of DTCA were formed on gold. Second, pSerMA brushes were synthesized via a method of SI-PIMP.

Antifouling performance of a surface depends not only on the intrinsic chemical properties of the coating material (i.e., molecular structure, and functional groups), but also the physical properties of the coating layer (i.e., film thickness, packing density, and uniformity), which are determined by the deposition method and conditions.[24] One of the most widely used living polymerization methods to graft polymer brushes on surfaces is the atom transfer radical polymerization (ATRP), which enables polymer films with uniform and well-controlled thickness and high packing density. ATRP has been used to graft a variety of antifouling polymers, such as pSBMA, pCBAA, and polyacrylamide, to achieve ultralow fouling surfaces.[17,19,22] However, ATRP has certain limitations due to its required usage of a transition metal catalyst. Some monomers can complex with the metal catalyst thus can hardly be polymerized

Scheme 2. Preparation of pSerMA on Gold via SI-PIMP



dx.doi.org/10.1021/bm301646y | *Biomacromolecules* 2013, 14, 226−231

via ATRP. Also the catalyst is toxic, therefore needs to be strictly removed before using the polymers for biomedical applications. The SerMA monomer used in this work was found to significantly complex with the transition metal catalyst (copper ion), and therefore cannot be polymerized on surfaces via ATRP. Instead, a SI-PIMP method was employed in our work to graft the pSerMA brushes on gold surfaces.

The SI-PIMP method has recently emerged as another effective approach to prepare polymer brushes with uniform and controlled thickness at high surface packing densities.[33,34] SI-PIMP is free of toxic catalyst, is not limited to specific types of monomers, and allows easy control over polymer films by varying light exposure temporally and spatially. Compared to the conventional surface-initiated polymerization by photoirradiation, SI-PIMP provides better control over the film thickness and uniformity of the polymer films.[33] The photoiniferter, which is different from a simple photoinitiator, acts as an initiator, chain transfer agent, and terminator, thus allowing better control over the polymerization reaction. SI-PIMP has been previously used to graft pCBAA and poly(*N*-isopropylacrylamide) from gold surfaces, with the film thickness controlled by the UV-irradiation time.[33,34]

It is generally accepted that the film thickness of the antifouling polymer brush layer is a key factor affecting protein adsorption.[24] With SI-PIMP, polymer film thickness can be easily controlled by varying the photoirradiation time (i.e., the polymerization time). Therefore, the relationship between the pSerMA film thickness and UV-irradiation time was first studied. Figure 2 shows that the pSerMA film thickness increased linearly initially and leveled off after polymerization for 150 min, likely owing to the chain−chain termination reaction.

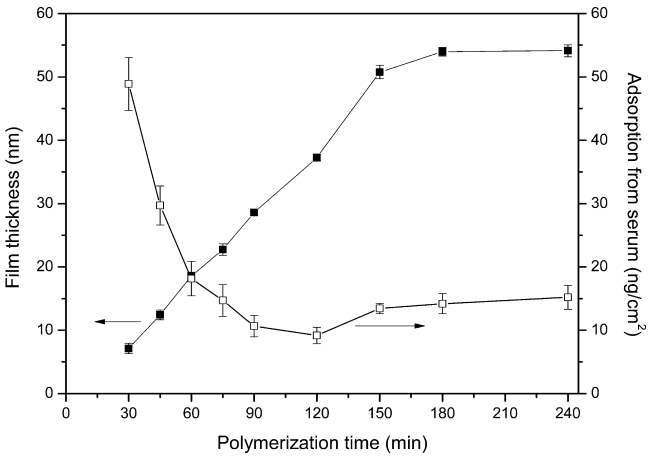


Figure 2. Thickness of the pSerMA films (solid square) and nonspecific adsorption from 100% blood serum onto the pSerMA-grafted surfaces (empty square), as a function of the polymerizaton time.

Dependence of the nonspecific adsorption from blood serum onto the pSerMA-grafted surfaces as a function of the UV-radiation time was also shown in Figure 2. For the thin pSerMA films, surface resistance to protein adsorption was limited, due to insufficient surface hydration. As the film thickness increased, the polymer brushes suppressed protein adsorption more effectively. A minimal adsorption of 9.2 ng/cm^2 from serum was found at the UV-irradiation time of 120 min, with a film thickness of 37.2 nm. Further increase of the polymer film thickness, however, led to more protein adsorption. Considering the zwitterionic nature of pSerMA, when polymer coatings became too thick, the long polymer chains may conformationally self-condense via inter- or intramolecular electrostatic interactions. As a result, the polymer−water interactions were decreased, leading to weaker surface hydration and increased protein adsorption. As shown in Table 1, the pSerMA film with a polymerization time of 120 min displayed a minimal water contact angle, indicating that the strongest surface hydration occurred at a medium film thickness, which correlates well with the protein adsorption results. Similar film thickness effects were observed for other antifouling materials such as pSBMA,[19] pCBAA,[22] and polyacrylamide.[17] From now on, unless otherwise specified, the UV-irradiation was conducted with the optimal time of 2 h to prepare the pSerMA-grafted samples.

To verify whether the grafted polymer film was uniform, the film thickness at different locations along the length of the chip was characterized by ellipsometry. Very small changes of the localized film thickness along the substrate, as shown in Figure 3, prove that uniform pSerMA brushes were achieved by the method of SI-PIMP.

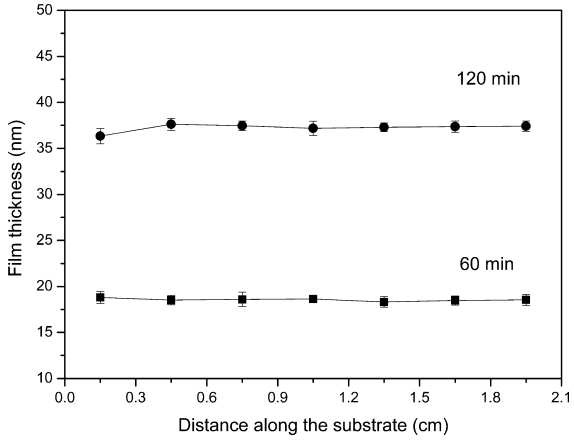


Figure 3. Polymer film thickness at different locations along the length of the substrate (~ 2.5 cm long) with different UV-irradiation times.

Protein resistance properties of the pSerMA brushes were evaluated by SPR. BSA was used as a model protein to study the single protein adsorption. Figure 4 shows that the adsorption of BSA on pSerMA brushes was only 1.8 ng/cm^2, much lower than the adsorption level of 83 ng/cm^2 on the bare gold surfaces.[17] Previous work by Shiraishi et al. reported that the p(SerMA-*co*-MMA) microspheres had BSA adsorption of ~34 ng/cm^2, 56% lower as compared to 78 ng/cm^2 for the PMMA microspheres.[25] It is demonstrated by this work that with well-controlled physical properties (e.g., film thickness) zwitterionic pSerMA can significantly suppress protein adsorption to a very low level. Notice that the isoelectric

Table 1. Water Contact Angle of the pSerMA-Grafted Surfaces

grafting time (min)	0	30	45	60	90	120	150	180	240
contact angle (deg)	80 ± 1	53 ± 2	46 ± 1	43 ± 1	36 ± 1	29 ± 2	31 ± 1	37 ± 2	39 ± 2

dx.doi.org/10.1021/bm301646y | *Biomacromolecules* 2013, 14, 226−231

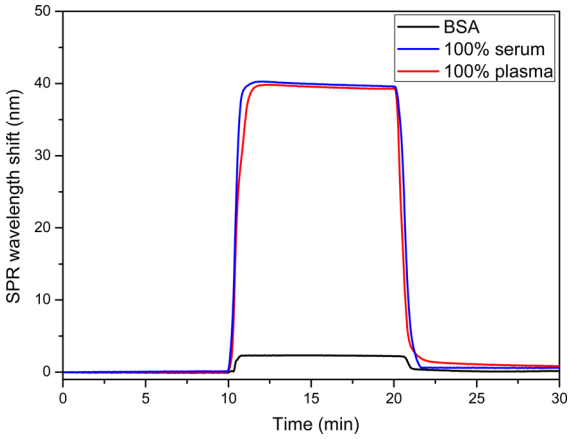


Figure 4. Protein adsorption on the pSerMA-grafted gold surfaces from 1 mg/mL BSA, 100% human blood serum, and 100% human blood plasma.

point of SerMA is 5.08,[25] while serine has the characteristic pK_1 of 2.21 (for −COOH) and pK_2 of 9.15 (for $-NH_3^+$). At the pH value of 7.4, the majority of the pSerMA brushes are in the zwitterionic status ($H_3N^+-C-COO^-$), while overall the polymer brushes are slightly negative.

Human blood serum and plasma contain hundreds of highly concentrated proteins. Compared to the single protein adsorption, resistance of antifouling materials to these complex media are much more challenging.[24] Figure 4 shows typical SPR spectra of protein adsorption from pure human serum and plasma onto the pSerMA-grafted surfaces. The average nonspecific adsorption from 100% serum and 100% plasma were 9.2 (±1.3) and 12.9 (±2.1) ng/cm^2, respectively. In contrast, protein adsorptions from serum or plasma onto the bare gold or photoiniferter SAM-modified gold surfaces were all above 150 ng/cm^2 (Figure 5). It should be noted that the

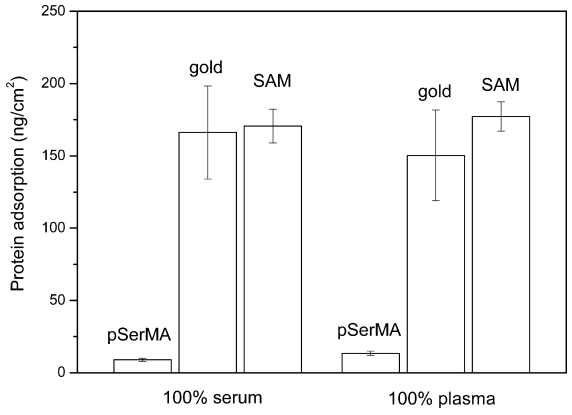


Figure 5. Protein adsorption of 100% human blood serum and 100% human blood plasma on the pSerMA-grafted gold, bare gold, and photoiniferter SAM-modified gold surfaces, evaluated by SPR.

pSerMA brushes in this study showed comparable or even better protein repellent property compared to some other antifouling materials such as the poly(β-peptoid) pEtA20,[16] poly(oligoethylene glycol methacrylate) (pOEGMA),[35] and poly(hydroxypropyl methacrylate) (pHPMA),[18] which were exposed to the same complex media under identical SPR conditions. The pEtA20, pOEGMA, and pHPMA surfaces had adsorptions of 10.8, 30, and 24.5 ng/cm^2 from serum, and adsorptions of 9.8, 10, and 52.8 ng/cm^2 from plasma, respectively.[16,18,35]

Cell attachment onto the pSerMA-grafted surfaces was finally investigated. Endothelial cells were cultured with the pSerMA-grafted or uncoated gold surfaces in culture medium (with 10% FBS) for 7 days. No cells were observed on the pSerMA-grafted surfaces after culturing for one week (Figure 6). In contrast,

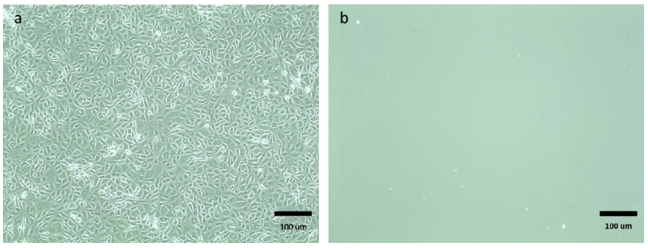


Figure 6. Cell attachment on (a) uncoated gold and (b) pSerMA-grafted gold surfaces after culturing for 7 days.

cells quickly attached, proliferated, and formed confluent monolayers on the uncoated gold surfaces. The work demonstrates that the pSerMA brushes not only suppress protein adsorption, but also resist cell adhesion. Similar cell repellence and correlation between protein suppression and cell resistance have been reported for other antifouling surfaces such as pSBMA, pCBMA, pHPMA, and polyacrylamide.[17,18,20,21,36]

Besides being antibiofouling, pSerMA has other attributes, such as the biomimetic nature of amino acid and the existence of multiple reactive surface groups which allows for further conjugation. The amino acid-based zwitterionic pSerMA is expected to be a promising antifouling and multifunctional material for a wide range of applications such as coatings for biomedical implants, drug delivery carriers, and biosensors.

■ CONCLUSIONS

Zwitterionic serine-based pSerMA brushes with uniform and well-controlled thickness was achieved on gold surfaces via SI-PIMP. With the optimal polymer film thickness, pSerMA-grafted surfaces can strongly suppress not only single protein adsorption, but also adsorptions from full human blood serum and plasma. The grafted surfaces also highly resist mammalian cell attachment. PSerMA can be considered an effective alternative to the traditional ethylene glycol-based antifouling materials.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Prof. Shaoyi Jiang at the University of Washington for providing SPR chips and Prof. Stephen Z. D. Cheng for the use of ellipsometer. We appreciate supports from the Cleveland Clinic Foundation/Clinical Tissue Engineering Center

(TECH-09-006A), Firestone Research Initiative Fellowship, and the University of Akron Faculty Research Grant.

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