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Protein-resistance performance of amphiphilic copolymer brushes consisting of fluorinated polymers and polyacrylamide grafted from Silicon surfaces

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Abstract: A series of random copolymer brushes of acrylamide (AM) and 2-(perfluorinated hexyl) ethyl methacrylate (FMA) were grafted from initiator-functionalized silicon wafers by surface-initiated atom transfer radical polymerization. The water contact angle, X-ray photoelectron spectroscopy and atomic force microscopy were used to characterize surface wettability, surface composition, morphology of the surfaces modified with polymer brushes, respectively. The protein-resistance performance of the surfaces grafted with polymer brushes was evaluated by micro-BCA protein assay reagent. It was found that the random copolymer brushes with the optimal ratio of AM to FMA in monomers showed the

best protein-resistance performance, however, the optimal ratio is different for resisting bovine serum albumin and human plasma fibrinogen adsorption, respectively. For resisting bovine serum albumin adsorption, the optimal ratio of AM to FMA in monomers is 1:3, while the optimal is 3:1 for human plasma fibrinogen adsorption resistance. The results provide further evidence that surface compositional heterogeneities and microphase segregation of fluorinated moieties of amphiphilic random copolymer brushes significantly impact protein adsorption behaviors.

Keywords: surface grafting; fluorinated copolymer brushes; amphiphilic polymer brushes; protein adsorption;

Introduction

Biofouling is one of the most significant limitations to the final applications of many biomaterial systems.¹ Nonspecific protein adsorption on biomaterials is an important factor of causing biofilm formation and consequent biofouling.² It is critical to develop materials to prevent nonspecific protein adsorption for many applications, such as biomedical diagnostics, tissue engineering, drug carriers, biosensors and marine coatings, etc.³ Protein adsorption is a complex process, mainly influenced by three factors: the nature of proteins, solution conditions (adsorption environment), and surface properties. Among of them, surface properties (including surface composition, surface morphology, and surface structure) can be transformed by proper regulation,

which have received more attention. So far, there are following several kinds of materials for resisting protein adsorption: hydrophilic materials, zwitterionic materials, fluorinated polymers, and amphiphilic materials. Poly(ethylene glycol) (PEG)⁴⁻⁶ is one of the most widely investigated hydrophilic resistant-protein materials. It can inhibit protein adsorption because of the hydration layer creating via hydrogen bonds, which constructs a steric barrier to adsorption. PEG loses its protein-resistance performance in the presence of oxygen and transition metal ions or above 35 °C. As an alternative, super-low fouling zwitterions materials reported by Jiang, et al,⁷⁻⁹ showed better performance resulting from forming a hydration layer via electrostatic interactions. It is also believed that lower surface energy plays an important role in surface resistance to protein adsorption. Hence, fluorinated polymers with low surface energy such as fluorinated polyimide^{10,11} and fluorinated poly(methylmethacrylate) (PMMA) copolymers,¹² have been used as resistant-protein materials. It is generally considered that fluorinated polymers with low surface energy can greatly reduce the polar and hydrogen bonding interactions with the proteins and fouling organisms or result in the feasible release of the proteins from the surfaces.^{12,13} In addition, amphiphilic polymers such as, polystyrene (PS)/PEG, PMMA/ poly(ethylene glycol) dimethacrylate (PEGMA) and fluorinated polymers/PEG.¹⁴⁻¹⁶ exhibit excellent performance in resisting protein adsorption attributing to the surfaces with morphological, topological and compositional complexity.^{17,18}

Amphiphilic polymers with excellent antifouling performance increasingly attract

the attention of researchers. However, the antifouling amphiphilic surface coatings reported in previous references were obtained from the films of block or random copolymers of fluorinated polymers and hydrophilic polymers, such as perfluoroether-PEG,^{17,19,20} P(PEGMA-co-MMA)-b-PC₆SMA,²¹ and amphiphilic homopolymers containing hydrophilic and hydrophobic groups. The amphiphilic films were prepared by spin-coating technique, casting technique and melting of copolymer grains onto the solid substrates.¹² It was found that the protein adsorption resistance of the amphiphilic surfaces were affected significantly by the factors of film preparation technique and film formation conditions, except for the chemical structure of the polymers.²²⁻²⁴ The amphiphilic films mentioned above were rough and nonuniform. More importantly, they are easy to fall off from the substrates, which limits their long-term utility.

The aim of this work is to exploit stable, smooth, uniform and long-lasting amphiphilic materials as resistance protein adsorption. The copolymers of poly(AM-ran-FMA) brushes were grafted from initiator-functionalized silicon wafers by surface-initiated atom transfer radical polymerization (SI-ATRP). AM and FMA as monomers were chosen to synthesize the copolymer brushes through covalent bonds linked to the silicon wafers for several reasons as follow: i) PAM has strong resistance protein adsorption²⁵⁻²⁷ and it is a biocompatible, electrically neutral, stable and water soluble polymer.²⁵ ii) FMA has lower surface free energy and chemical durability. iii) The mixtures of AM and FMA can copolymerize to obtain PAM-ran-PFMA brushes

because the two monomers have similar polymerization conditions. Finally, a series of random copolymer brushes and corresponding homopolymer brushes were successfully grafted from the silicon surfaces. The influence of surface composition, surface structure and the segregation of the fluorinated moieties in the performance of resisting protein adsorption were further investigated.

Experimental section

Materials and Reagents.

Silicon wafers [n-doped, (100)-oriented, 0.56 mm thick, 100 mm diameter one side polished, were supplied by Wacker Chem tronics (Germany) and cut into 1 cm×1 cm samples. 3-Aminopropyltriethoxysilane (APTES), bromoisobutyl bromide (BIBB 99%), ethyl 2-bromoisobutyrate (EBIB 99%) and the ligand N,N,N',N'',N'''-Pentamethyldiethylenetriamine (PMDETA 99%) were purchased from Aladdin Chemistry Co.Ltd. 2-(perfluorinated hexyl) ethyl methacrylate (FMA 95%) was supplied by Matrix Scientific. The acrylamide monomer (AM, 98.5% Shanghai Chemical Reagent Co.). AK-225(Asahi Glass Co.). All the reagents mentioned above were used as received. Catalyst copper(I) bromide (CuBr, Shanghai Chemical Reagent Co. ≥97%) was purified by stirring in acetic acid, washing with ethanol and drying under vacuum. Triethylamine (TEA) and all other solvents were purchased from Shanghai Chemical Reagent Co. and purified according to standard methods before use. Deionized water purified by a Millipore water purification system to give

a minimum resistivity of 18.2 M Ω ·cm was used in all experiments. Argon gas was of high-purity grade. Bovine serum albumin (BSA) and Human plasma fibrinogen (Fg) were purchased from Sigma Chemical Co. (St. Louis, MO) and used without further purification. Phosphate buffered saline (PBS, pH, 7.2-7.4; ion strength, 0.01 M). Reporter Lysis 5 $\times$ Buffer (Promega USA), five times dilute with PBS as used.

Surface Preparation and Modification

Surface treatments. The silicon wafers were treated with a hot “piranha” solution (7/3 volume ratio, 98% H₂SO₄/30% H₂O₂) (*Caution: Piranha solution reacts violently with organic materials, and should be handled with care!*). Followed by ultrasonic rinsing with water four times and drying in oven at 60 °C to obtain a hydroxyl-terminated Si surfaces.

Synthesis of Amino-Functionalized Silicon Wafers. The freshly prepared hydroxyl-terminated silicon wafers were immersed in a toluene solution (2.5 vol%) of APTES at room temperature overnight. After that, the amino-functionalized silicon substrates were ultrasonically washed successively with toluene, dichloromethane, ethanol, and water and then dried in an argon stream.

Synthesis of Initiator-Functionalized Silicon Wafers. The freshly prepared amino-functionalized silicon wafers described above were soaked in a solution of 0.1 mL of TEA (0.7 mmol) in 20 mL of dry dichloromethane. After the samples had cooled to 0 °C, BIBB (0.5 mL, 4.04 mmol) was added drop-wise into the mixture. The reaction was performed at 0 °C for 2 h and then at room temperature for 12 h. The

obtained initiator-functionalized silicon wafers were cleaned with ultrasonic successively with toluene, dichloromethane, ethanol, and water and then dried in an argon stream.^{28,29}

Preparation of PAM and PFA Copolymer Brushes The copolymers of poly(AM-ran-FMA) grafted from typical procedure for initiator-functionalized silicon wafers by SI-ATRP. Initiator-functionalized silicon wafers, PMDETA (57 μ L, 0.276 mmol), monomers (10.35 mmol sum of AM and FMA), initiator EBIB (20 μ L 0.138 mmol) and 15 mL solvent DMF distilled by reducing pressure were added separately to glass tubes those were capped with a rubber septum and equipped with a magnetic stir bar. The mixture was deoxygenated by four freeze-pump-fill argon-thaw cycles. In the second cycle CuBr (20 mg, 0.138 mmol) was added to the mixtures under the protection of argon. The mixture in the tube filled with argon was kept in the oil bath at 110 °C for 72 h with stirring, and then exposed to air to stop the polymerization. The silicon wafers grafted with polymer brushes were ultrasonic washed repeatedly two times with AK-225, ethanol and water and then dried in an argon stream. The series copolymer brushes were obtained by adjusting the mole ratio of AM to FMA with 3:1; 1:1, and 1:3. Moreover, the homopolymer brushes of PAM and PFMA were prepared by the same method. The experiment flow chart is shown in **Scheme 1**. The obtained different samples number according to the monomer proportion is shown in Table S1. (Supporting Information)

Characterization

Water contact angle measurement The wettability of silicon surface modified with grafted-layer was evaluated by measuring the contact angle of de-ionized water (WCA) by using a Contact Angle measuring System (OCA20, Dataphysics Instruments with GmbH, Germany). A water droplet of 2 μL was used with the sessile drop method at ambient temperature. Each reported value is the average of nine measurements taken from different locations of the surface .

X-ray photoelectron spectroscopy (XPS) The chemical composition of the surfaces modified by copolymer brushes was analyzed by (KRATOS XSAM800) using Mg K (1253.6 eV) as radiation source. The take-off angle of the photoelectron was set at 90° and binding energies were calibrated by using the containment carbon (C 1s=284.6 eV). Survey spectra were run in the binding energy range 0–800 eV and the spectra of Br 3d, C 1s, N 1s, O 1s and F 1s were collected.

Atomic force microscope (AFM) An atomic force microscope (multimode 8 microscope, Bruker, USA) was used to detect the surface topography. The surface morphology can be seen in the AFM images acquired in the Scanasyst Air mode with silicone tip cantilevers having a nominal spring constant of 0.4 N/m, frequency of 70 kHz. The roughness of surfaces was evaluated by the value of Ra (mean surface roughness), which was determined from $2.0\ \mu\text{m} \times 2.0\ \mu\text{m}$ area of the surface and was the average of three measurements taken from different regions.

Protein adsorption

BSA and Fg were used as model proteins to evaluate the protein-adsorption resistance

of silicon surface with modification by PAM-ran-PFMA copolymer with different fluorine content and corresponding homopolymer brushes. The adsorption experiments were performed by BCA protein assay reagent³⁰⁻³². Polymer-modified silicon wafers with 1 cm×1 cm area were immersed in PBS solution containing 2 mg/mL BSA or 1 mg/mL Fg. The adsorption was incubated in shaker at a constant temperature of 37 °C for 24 h. After adsorption, the samples were taken out and rinsed with PBS three times to remove the non-adsorbed protein. And then, the remaining protein adsorbed on the surface was detached in 1 ml Reporter Lysis 1× Buffer by ultrasonic washing for 30 min at room temperature. The Micro BCA protein-analysis kit (no. 23235, Pierce, Rockford, IL) based on the bicinchoninic acid (BCA) method was used to determine the concentration of the protein in Reporter Lysis 1× Buffer by measuring the absorbance at 562 nm by UV-Vis measurement with a Shimadzu UV-2200 spectrophotometer.³¹ The amount of adsorbed protein per unit area was calculated based on the concentration of protein and pre-determined calibration curve obtained by measuring the absorbance of 0–2 mg/ml protein solutions in Reporter Lysis 1×Buffer. And then, the amount of protein adsorbed onto each samples was calculated, to obtain the reported data, which is the average of three parallel measurements.

Results and Discussions

Characterization of silicon surface modified with initiator and polymer brushes.

The WCA of Si-OH, Si-NH₂, Si-initiator and a series of samples from S1 to S5 modified with polymer brushes was shown in Figure 1. The contact angle of hydroxyl-terminated silicon wafer was 15 degree and then increased to 40° after immobilization of APTES, indicating APTES was successfully anchored onto the hydroxyl-terminated silicon wafer. The WCA of initiator-functionalized silicon wafers increased to 70° from 40° similar to the reported value.³³ After the modification of PAM brush on the surface, the CA decreased to 33. The changes of the WCA from before and after the modification confirmed the formation of copolymer brushes on the silicon surfaces. With the increase of FMA mole percentage in monomers, the WCA of the copolymer brushes increased rapidly and approached the value of PFMA homopolymer brushes when the molar ratio of AM/FMA in monomers was more than 1:3, which agrees with the results in previous literature.³⁴ It is generally believed that the surface wettability is determined by surface composition and surface morphology, characterized as follows.

The XPS spectra of amino-functionalized silicon wafers (Si-NH₂) and initiator-functionalized silicon wafers (Si-initiator) were displayed in **Figure 2**. There is no signal of Br for the surface of Si-NH₂, while for the surface of Si-initiator there is a signal of Br at 69 eV, which is attributed to Br from the initiator. This result indicated that initiator was successfully anchored onto amino-functionalized silicon wafers. The XPS spectra of silicon wafers grafted with PAM and PAM-ran-PFMA were shown in Figure 3. C, N and O signal peaks were detected from Si-PAM and the

ratio of C/O/N is 60.9/18.4/20.6 (shown in Supporting information Table S1.), close to estimated value (3/1/1) of PAM based on the molecular formula, indicating the surface was successfully grafted with PAM brushes. In the XPS spectra of Si-(PAM-ran-PFMA), a signal peak appeared at 690 eV attributing to F 1S, which confirms that PAM-ran-PFMA brushes were successfully immobilized on silicon surfaces. The XPS spectra of the surface modified with different ratio of AM/FMA were shown in Figure S1. C, N, O, F signal peaks were detected in the XPS spectra of S₂, S₃, S₄ and S₅ shown in **Figure S1**. The relative content of fluorine was 28.8 (at %), 44.1 (at %), 43.1 (at %) for copolymer brushes S₂, S₃, S₄, respectively and was close to the peak value 45.3 (at %), the value of fluorine content for PFMA homopolymer brushes, when the molar ratio of AM/FMA in monomers was higher than 3:1, as shown in **Table S1**. From the data can be seen, the tendency of fluorine content on the surface were similar to the contact angle variation trends mentioned above, proving the effect of surface composition on the surface wettability.

To further investigate the composition of functional groups on the surfaces, the XPS C1s spectra of surfaces, shown in **Figure 4**, were recorded by deconvolution software and curving fitting technique. The discrete peaks were assigned to different carbon-based functional groups, and their corresponding content can be calculated from the relative peak areas of the individual carbon components. The C1s spectra of Si-initiator and PAM-grafted surfaces were composed of three Gaussian curve-fitted peaks: a C-C component at 284.8 eV due to aliphatic hydrocarbons, a C-N component

at 286.0 eV, and a C=O component at 288 eV.^{23,35} The relative content of the same element on above-mentioned two surfaces are different, displayed in **table 1**, because they derive from different functional groups. For instance, the peak of C-N (45.8%) in **Figure 4-S₀** is from the bond between amino of silane and initiator, and peak of C-N (57.5%) in **Figure 4-S₁** is mainly attributed to amide carbon O=C-N. As expected, the relative content of C-N in high-resolution C 1s spectra increased from 45.8% to 57.5%, resulting from the surface covered with a PAM layer. The C1s spectra in **Figure 4-S₂**, **Figure 4-S₃**, **Figure 4-S₄**, and **Figure 4-S₅** were resolved into five Gaussian curve-fitted peaks: hydrocarbon (C-C) around 284.6 eV, C-O-C and C-N around 286.5 eV, C=O around 288.5 eV, CF₂ around 291.5 eV and CF₃ around 294.0 eV, from low to high binding energy respectively. The peak assignments are basically consistent with the previous reports.²³ Comparing with C_{1s} core level region in **Figure 4-S₀** and **Figure 4-S₁**, C_{1s} core level region of the fluorinated polymer brushes (S₂, S₃, S₄ and S₅) is added with two Gaussian curve-fitted peaks, CF₂ around 291.5 eV and CF₃ around 294.0 eV, coming from PFMA. This is further evidence that polymer brushes of PFMA were grafted onto silicon wafer surfaces successfully, according with the analysis result of WAC and F relative content. Moreover, C-F bonds come from CF₃ head groups and a sequence of five CF₂ groups. The two groups and their orientation have different contributions to surface properties, such as surface energy and protein-resistant properties.^{24,36} **Figure 4** and **Table 1** display the ratio of CF₃/CF₂ is different with the difference of molar ratio of AM/FMA in monomers. It is obvious

that the ratio of CF_3/CF_2 increases with the increase of FMA in monomers, which illustrates that the arrangement of fluorinated groups in the copolymer brushes strongly depends on the mole percent of FMA in monomers. When the ratio of AM to FMA is 3:1, the C-F bonds on the surfaces detected almost all come from CF_2 , meaning that the fluorine-containing side chains are parallel to the substrates. In other words, with the increasing of FMA mole percent in monomers, the segregation of CF_3 onto the surfaces increases, which implies there are more fluorine-containing side chains perpendicular to the substrates.^{24,37}

The morphology of surfaces after modification with polymer brushes was studied with AFM and shown in **figure 5**. The average roughness value (Ra) of Si-initiator was 2.6 nm for a $2\ \mu\text{m} \times 2\ \mu\text{m}$ scan area and increased to 4.8 nm and 4.1 nm after grafting PAM and PFMA homopolymer brushes. And the Ra of copolymer brushes are 3.9 nm, 3.4 nm and 3.4 nm corresponding to monomer molar ratios of AM to FMA are 3:1, 1:1 and 1:3, respectively. It indicates that the surfaces grafted with homopolymer and copolymer brushes have similar roughness and they are more rough than Si-initiator surface. The topographies, accompanying line profiles of surface of S_3 and the method of calculating the height difference between silicon surface (100) and silicon surface after modification with polymer brushes, were shown in **Figure 6**. The high lighting is the evolution of surface morphologies of the silicon wafer after modification with polymer brushes and the black area is background of Si (100). The Z range of the scans in S_1 , S_2 , S_3 , S_4 and S_5 surface S_0

cases is 7.4 nm, 10.7 nm, 10.3 nm, 10.3 nm, and 10.6 nm, respectively. That is, the thickness of polymer brushes grafted onto the surfaces are 7.4 nm, 10.7 nm, 10.3 nm, 10.3 nm and 10.6 nm, respectively, shown in **Table S1** (in Supporting Information). As can be seen from above data, the thickness and roughness of grafted layers have a small difference.

The results of WCA and XPS indicated that the polymer brushes were successfully grafted from silicon surfaces. AFM measurements showed that the PAM and PFMA homopolymer and copolymer brushes grafted from silicon surfaces have similar thickness and roughness.

Protein adsorption

Adopting the widely used BSA and Fg as indicative substance, the resistant-protein performance of the polymer-grafted surfaces was evaluated by micro-BCA protein assay reagent. BSA (Mw=66 kD; pI=4.9) is a common carrier globulin and has a larger solubility in water. Fg (Mw=340 kD; pI=5.5), a blood coagulation factor, is one of the most relevant protein adsorbed biomaterials surfaces. They are the most representative non-specific adsorption proteins with different sizes, molecular structure.^{35,38} The assay results of resistant-BSA adsorption were displayed in **Figure 7**. The amount of BSA adsorbed on bare silicon surface is much more than that on the surfaces grafted with polymer brushes, confirming the resistance BSA-protein adsorption of surfaces modified with both PAM, PFMA homopolymer brushes and PAM-ran-PFMA copolymer brushes. Moreover, the amount of BSA adsorbed on

polymer brushes is different with the difference of PFMA content in monomers owing to the different surface wettability, surface composition and segregation of fluorinated moieties, in the condition of aqueous solution at pH=7.4 and 37 °C.³⁹⁻⁴¹

The amount of BSA adsorbed on hydrophilic PAM brushes and hydrophobic PFMA brushes is neither the least nor the most, which indicates hydrophilicity or hydrophobicity is not resistant-protein-adsorption decisive factor. It is clear that the copolymer brushes with 1:3 monomer mole ratio of AM to FMA have the strongest resistance BSA adsorption probably owing to the combined action of surface hydration and segregation of fluorinated moieties. Comparing with hydrophobic PFMA brushes, the copolymer brushes with 1:3 monomer ratio of AM to FMA containing hydrophilic PAM, which forming a hydration layer can resisting protein adsorption in PBS solution. On the other hand, for copolymer brushes with 1:3 monomer ratio of AM to FMA, the ratio of CF₃ to CF₂ is 1:1, which may be a proper ratio to forming the best surface structure of molecular size scale for reducing the interaction between BSA and substrate.^{34,37,42}

The amount of Fg adsorbed on the surfaces of bare silicon and surfaces modified with polymer brushes is presented in **Figure 8**. It was displayed that the amount of adsorbed Fg on the surfaces modified with polymer brushes is much less than that on the surface of bare silicon, indicating that the surfaces grafted with PAM and PFMA homopolymer brushes as well as fluorine-containing copolymer brushes can reduce Fg adsorption. The amount of adsorbed Fg increases with the increase of the content

of FMA in monomers, while the amount of adsorbed Fg on the PAM brushes is more than that on copolymer brushes with the 3:1 mole ratio of AM to FMA in monomers, which is the optimum for resisting Fg adsorption. It can be explained that Fg is a rod-shaped protein with a symmetrical dimeric structure containing two sets of three intertwined polypeptide chains, which is easily adsorbed on the hydrophobic surface and exists in a flat-topped conformation.^{43,44} In addition, PAM polymer brushes adsorbed more Fg than copolymer brushes with 3:1 monomer mole ratio of AM to FMA because the copolymer brushes have both hydration layer and fluorinated moieties with low surface energy and the fluorinated moieties have smaller size than Fg, which resulting in stronger resistance to Fg adsorption.

The results and data reported in this work prove that the copolymer brushes exhibit the best protein-resistant property, however, for BSA and Fg the copolymer with best protein-resistant performance have different monomer molar ratio of AM to FMA. Namely, the minimum amount of different protein adsorbed appears in a certain different proportion of the surface functional groups composition.³⁴ The study proved further evidence that surface composition, protein molecular size scale and the pattern of microphase segregation in copolymer brushes⁴⁵ play an important role in protein adsorption behaviors under the thickness and roughness of polymer brushes are similar conditions, which is in agreement with the results reported.^{1,2,34}

Conclusions

In present work, PAM and PFMA homopolymer brushes and a series of random copolymer brushes were grafted from the silicon wafers and the surface composition, surface wettability, surface morphology and segregation of fluorinated moieties were characterized by using WCA, XPS and AFM. The properties of resisting BSA and Fg adsorption were evaluated by the means of BCA reagent. The surfaces grafted with polymer brushes including PAM and PFMA homopolymer brushes and random copolymer brushes were found to be repellant to BSA and Fg adsorption comparing to the surface of bare silicon. In addition, the best protein-resistance ratio of AM to FMA in monomers changed with different type of proteins. When the mole ratio of AM to FMA is 1:3, the surface has the strongest property of BSA-adsorption-resistance. while the optima mole ratio of PAM to PFM is 3:1 for Fg-adsorption-resistance. The results obtained in the study shows the resistant-protein performance of amphiphilic polymer brushes is influenced by surface composition, surface structure, and segregation of fluorinated moieties. In brief, the study provides repellant-protein amphiphilic fluorinated polymer brushes bound to the silicon surfaces via covalent bonds, which is stable, long-term, biocompatible and nontoxic. Besides, we investigated that surfaces composition, surface structure and segregation of fluorinated moieties greatly influence protein adsorption. The work provides novel resistance-protein surfaces modified with amphiphilic fluorinated copolymer brushes. The optimized copolymer brushes can resist BSA and Fg adsorption, respectively, which are promising in biomaterials and biotechnological applications.

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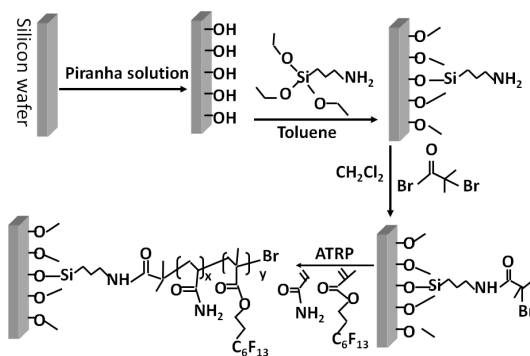
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Scheme1. Schematic representation of silicon wafer surface grafted with PAM-ran-PFMA brushes by SI-ATRP

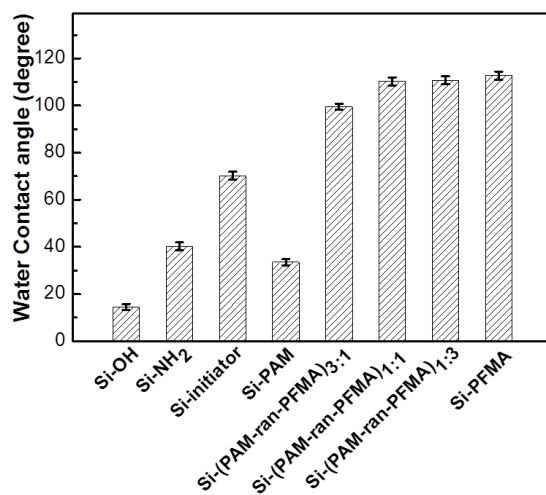


Figure 1. Contact angle measurement of surfaces modified with grafted layer.

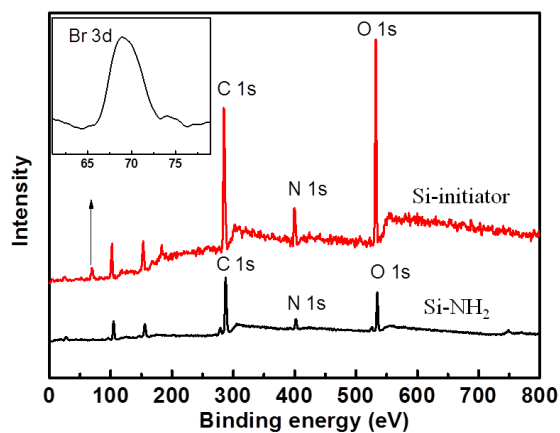


Figure 2. XPS survey spectra for the silicon surface after modification at a takeoff angle of 90°. Si-initiator and Si-PAM brushes.

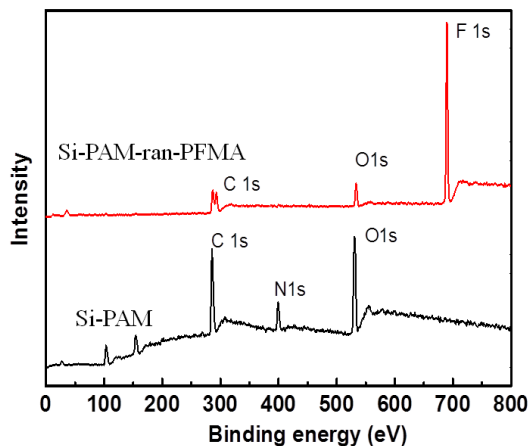


Figure 3. XPS survey spectra for the silicon surface after modification at a takeoff angle of 90°. Si-PAM and Si-(PAM-ran-PFMA).

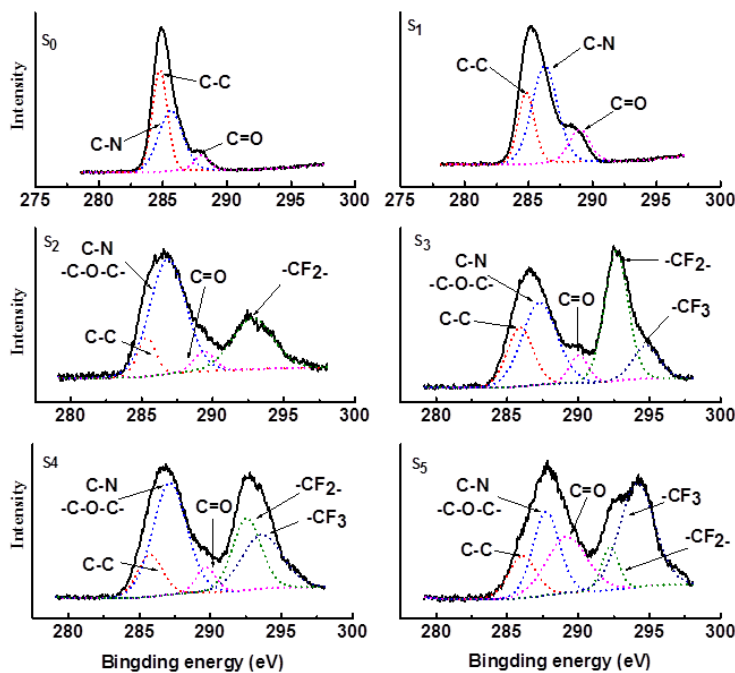


Figure 4. Area-normalized C (1s) XPS signal for the surfaces after modification at a 90° takeoff angle. S₀: Si-initiator, S₁: Si-PAM brushes, S₂: Si-(PAM-ran-PFMA) brushes with 3:1 mole ratio of AM to FMA in monomers; S₃: Si-(PAM-ran-PFMA) brushes with 1:1 mole ratio of AM to FMA in monomers; S₄: Si-(PAM-ran-PFMA) brushes with 1:3 mole ratio of AM to FMA in monomers; S₅: Si-PFMA brushes.

Table 1. High-Resolution Carbon 1s XPS Data (Fraction of C1s Signal, %)

Sample	Fraction of C 1s Signal (%)					
	C-C(H)	C-N(or C-O-C)	C=O	CF ₂	CF ₃	CF ₃ / CF ₂
	(284.8 eV)	(286 eV)	(288 eV)	(291.5 eV)	(294 eV)	
S ₀	47.3	45.7	7.0	--	--	--
S ₁	10.23	57.5	32.27	--	--	--
S ₂	9.7	56.42	5.42	28.46	--	0
S ₃	17.28	32.47	5.76	34.68	9.82	0.28
S ₄	10.93	39.57	5.03	21.56	22.9	1.06
S ₅	11.53	22.16	22.67	7.36	36.28	4.93

S₀: Si-initiator; S₁: Si-PAM brushes; S₂: Si-(PAM-ran-PFMA) brushes with 3:1 mole ratio of AM to FMA in monomers; S₃:Si-(PAM-ran-PFMA) brushes with 1:1 mole ratio of AM to FMA in monomers; S₄:Si-(PAM-ran-PFMA) brushes with 1:3 mole ratio of AM to FMA in monomers; S₅:Si-PFMA brushes.

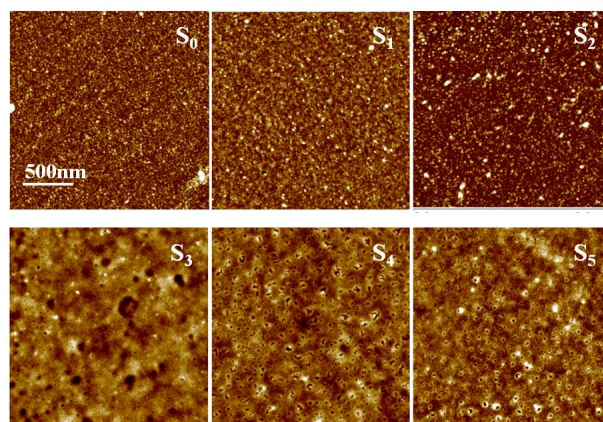


Figure 5. Scanasyt Air images (2 $\mu\text{m} \times 2 \mu\text{m}$), S₀:Si-initiator, S₁:Si-PAM brushes, S₂:Si-(PAM-ran-PFMA) brushes with 3:1 mole ratio of AM to FMA in monomers; S₃:Si-(PAM-ran-PFMA) brushes with 1:1 mole ratio of AM to FMA in monomers; S₄:Si-(PAM-ran-PFMA) brushes with 1:3 mole ratio of AM to FMA in monomers; S₅:Si-PFMA brushes. All samples were dried in a argon stream at room temperature.

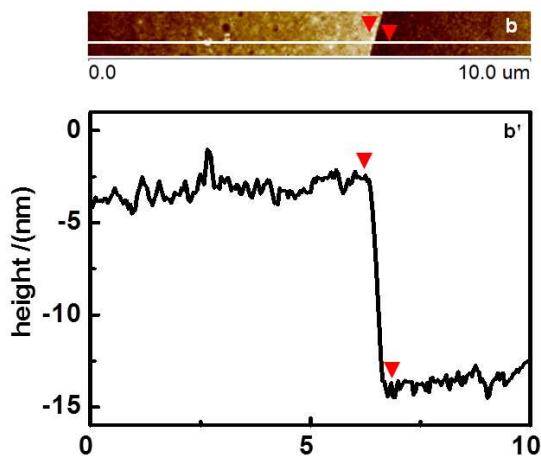


Figure 6. In situ AFM lithography for determining the height of polymer brushes on silicon wafer. b) The background is silicon wafer (100) and the white area in the topographic image is back-filled with initiator and polymer brushes. b') The cross-sectional analysis shows 10.4 nm height difference between silicon surface and polymer brushes. The value of relatively height difference of the rest of samples was obtained in the same method.

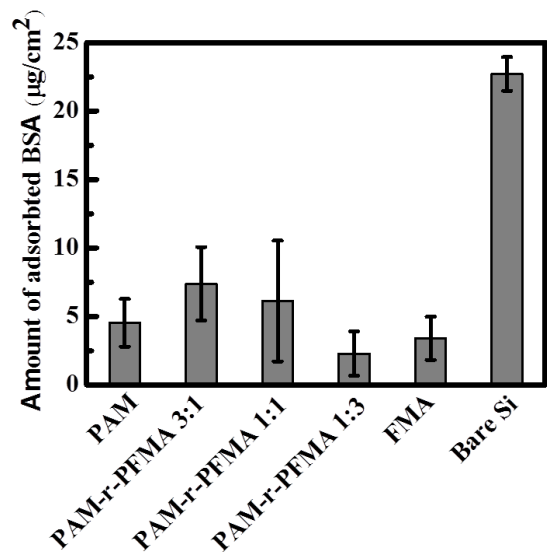


Figure 7. The amount of adsorbed of BSA onto the surfaces functionalized silicon wafers modified by copolymers brushes with different ratio of two monomers after 24 h incubation at an initial protein concentration of 2 mg/ml.

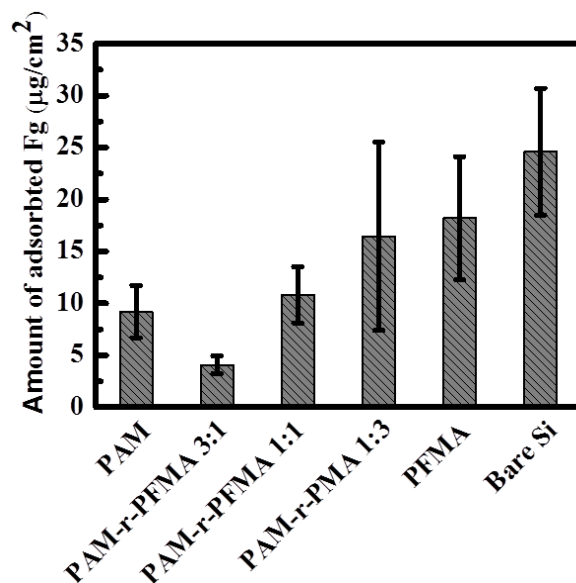


Figure 8. The amount of adsorbed of Fg onto surface functionalized silicon wafers modified by copolymers brushes with different ratio of two monomers after 24 h incubation at an initial protein concentration of 1 mg/ml.

TOC graph

