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Evaluation of Photochemically Immobilized Poly(2-ethyl-2-oxazoline) Thin Films as Protein-Resistant Surfaces

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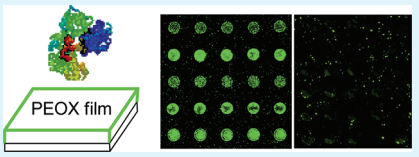
Supporting Information

ABSTRACT: Poly(2-ethyl-2-oxazoline) (PEOX) of various molecular weights were covalently immobilized on silicon wafers and gold slides to form protein-resistant surfaces via a fast and general photocoupling chemistry based on the CH insertion reaction of light-activated perfluorophenyl azide (PFPA). The thicknesses of the immobilized PEOX films ranged from 23 to 80 Å for molecular weight of 5000 to 500 000, and the grafting density reached 3.2×10^{-3} Å^{-2} for PEOX 5000. The protein-resistant property of the films was studied using bovine serum albumin (BSA) by fluorescence imaging, ellipsometry, and surface plasmon resonance imaging (SPRi). The fluorescence imaging and ellipsometry studies showed the largest amount of BSA adsorbed on PEOX 5000 and the smallest on PEOX 500 000. This was consistent with the kinetic analysis of BSA adsorption by SPRi showing that PEOX 5000 exhibited the fastest association rate and the slowest dissociation rate whereas PEOX 500 000 had the slowest association rate and the fastest dissociation rate. The PEOX film was then applied in the fabrication of carbohydrate microarrays to reduce the nonspecific adsorption of lectins and thus the background noises. Results showed that the microarray signals were significantly enhanced when the PEOX film was used.



KEYWORDS: poly(2-ethyl-2-oxazoline), thin films, protein-resistant surfaces, surface plasmon resonance imaging, carbohydrate microarray

INTRODUCTION

Searching for coating materials that offer biocompatibility as well as long-term stability is a continuous endeavor. Protein-resistant surfaces are highly desirable in bioanalysis and medical devices such as high-throughput assays and biosensors where the background noise caused by the nonspecific adsorption of proteins can effectively reduce the sensitivity and obscure the results.[1−5] In medical and diagnostic devices, a fouling surface attracts proteins and other biological species, which can lead to malfunction of the devices.[6,7]

Empirically derived design criteria indicate that hydrophilic, electrically neutral, and biocompatible polymers are among the best candidates having excellent protein-resistant properties.[8−12] Poly(ethylene glycol) (PEG), studied by numerous researchers, has demonstrated superb antifouling properties[1,11−18] and has thus become the "gold standard" and the most frequently used biocompatible material. However, PEG still has its limitations. Studies have shown that PEG can be slowly hydrolyzed in vivo via oxidative degradation,[19−21] an issue that limits its uses in situations where long-term stability is required.

Poly(2-oxazoline) (POX) and derivatives have attracted increasing attention recently, showing potential to be an alternative to PEG as an antifouling material.[22−24] Konradi,[25] Zhang,[26,27] and Hutter[28] have reported that POX-based polymer brushes showed a high protein-resistant property, which was similar to the best PEG-coated surfaces. In addition, POX is expected to be more stable against biological degradation than PEG because of its peptidomimetic structure rather than the polyether structure of PEG.[25,29]

Two strategies, the "graft-from" and "graft-to" methods, are generally used to covalently immobilize protein-resistant polymers. The "graft-from" approach, in which the polymer is synthesized in situ by polymerizing monomers from the surfaces, can be used to prepare high-density polymer brushes that often give better antifouling properties.[30] In the "graft-to" approach, the polymer is directly immobilized on the substrate by a surface coupling reaction. Typically, the polymer is derivatized with a functional group that can subsequently react with the substrate. For POX, functional groups are introduced either during the termination step in living cationic polymerization or by using a functionalized 2-oxazoline monomer. For example, Cesana et al. introduced thiol groups on POX by using 2-[2-(4-methoxybenzylsulfanyl)ethyl]-2-oxazoline as the monomer.[31] Jordan[32,33] and Rehfeldt[34] prepared trimethoxysilane-functionalized poly(2-alkyl-2-oxazoline)s via living cationic polymerization of 2-oxazolines followed by terminating the polymer with a monofunctional trimethoxysilane group. The trimethoxysilane-terminated polymers were then grafted to silicon wafers by silanization.

We developed a graft-to approach to covalently immobilize polymers without the need to functionalize the polymers.[35−40] The method is based on PFPA, which upon light or thermal activation generates the highly reactive singlet perfluorophenyl nitrene that can form covalent bonds with neighboring molecules

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via CH insertion or C=C addition reactions.[39,41,42] The coupling chemistry is applicable to a wide range of molecules and materials including those that lack reactive functional groups such as polyolefins,[43] and carbon materials of C_{60}[44] and graphene.[45] For polymers, the immobilization can be readily accomplished by coating the polymer on PFPA-functionalized surface followed by a fast light activation under ambient conditions. No prior derivatization of the polymer is necessary. Furthermore, light can be directed to designated regions on the sample creating spatially addressable polymer films in the areas of interest. These benefits are especially appealing in microarray and device applications where the antifouling property in conjunction with simple fabrication process offers attractive advantages over other techniques that involve chemical derivatization or in situ polymerization.

In this article, we employed the photoimmobilization chemistry to fabricate PEOX films on silicon wafers, glass slides and Au films, substrates that are widely used in microarrays and analytical devices. PEOX of varying molecular weights were studied, and the films were characterized by ellipsometry and dynamic contact angle goniometry. The protein-resistant properties of the immobilized films were evaluated by treating the films with BSA, a protein that adheres to many surfaces nonspecifically.[46] The extent of BSA adsorption was evaluated using ellipsometry and fluorescence imaging. The kinetics of BSA adsorption was also investigated by SPRi. Finally, the method developed was applied to the fabrication of carbohydrate microarrays where PEOX was used as the antifouling surface to reduce the background noise.

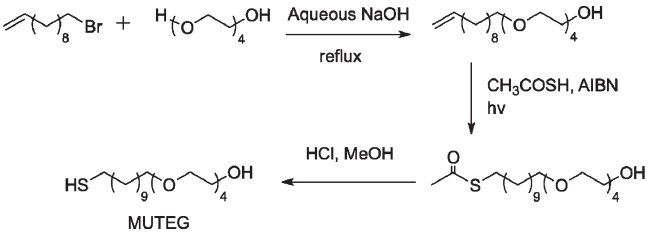
EXPERIMENTAL PROCEDURES AND METHODS

Materials. Milli-Q water for contact angle measurements as well as for cleaning gold slides and silicon wafers was obtained from a Millipore Milli-Q system with at least 18.2 MΩ resistivity. Concentrated H_2SO_4, H_2O_2 (35%), toluene, dichloromethane, and chloroform were purchased from Fisher Scientific. Ethanol (95%) was purchased from Aaper Alcohol & Chemical Co. (Shelbyville, KY). Dichloromethane was dried by refluxing in CaH_2 for 3 h and was distilled before use. Other solvents were used as received. 2,2′-Azobis(2-methylpropionitrile) (AIBN), polystyrene (PS, Ave. M_w *ca* 280,000), PEOX (Ave. M_w ca. 50 000 and 200 000, PDI 3−4), and poly(allylamine) (PAAm) hydrochloride (Ave. M_w ca. 70 000) were used as received from Aldrich. PEOX (Ave. M_w ca. 5000 and 500 000, PDI 3−4) were obtained from Alfa Aesar. The PEOX polymers were used as received without further purification. BSA (96%, ~66 kDa), fluorescein isothiocyanate-conjugated BSA (FITC-BSA), fluorescein isothiocyanate-conjugated Con A (FITC-Con A, lectin from *Canavalia ensiformis*, Type IV), phosphate buffered saline (PBS, pH 7.4), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (HEPES, pH 7.5), calcium chloride, and magnesium chloride were purchased from Sigma. 3-Glycidyloxytrimethoxy silane (GOPTS), D-(+)-mannose (Man), D-(+)-glucose (Glc), D-(+)-lactose (Lac), and dextran 40 000 were obtained from TCI. 2-*O*-α-D-Mannopyranosyl-D-mannopyranose (Man2) was obtained from V-laboratories, Inc. (Covington, Louisiana).

Silicon wafers with a 35 Å native oxide layer were purchased from WaferNet, Inc. (San Jose, CA). Glass slides (3 in. × 1 in. × 1 mm) were obtained from Corning Glass Works, Scientific Glassware Department (Corning, NY). The long-pass optical filter (280 nm) and high refractive index N-SF10 glass slides (18 mm × 18 mm × 1 mm) were purchased from Schott Glass Technologies, Inc. (Fullerton, CA).

PFPA-silane[35,37] and PFPA-disulfide[47] were synthesized according to previously published procedures. The synthesis of (1-mereaptoundec-11-yl)-tetra(ethylene glycol) (MUTEG) and the preparation of MUTEG SAM on gold slides followed the Pale-Grosdemang's method.[48] Briefly, monoetheration of tetra(ethy1ene glycol) with 11-bromo-1-undecene by refluxing in NaOH afforded the vinyl alcohol (Scheme 1). Photochemically initiated addition of thioacetic acid gave the thioester, which was then converted to MUTEG by refluxing with HCl in methanol (Scheme 1). All compounds were freshly prepared and purified using a silica-gel column before they were used to treat the silicon wafers or gold slides.

Scheme 1. Synthesis of MUTEG



Immobilization of PEOX and PS on Silicon Wafers. Silicon wafers were cut into 1 × 1 in. pieces, cleaned in the piranha solution (3:1 v/v conc. H_2SO_4/H_2O_2) at 80−90 °C for 1 h (Caution: the piranha solution reacts vigorously with organic solvents.), washed in boiling water three times for 60 min each, and then dried carefully under a stream of nitrogen. The cleaned wafers were soaked in a solution of PFPA-silane in toluene (12.6 mM) for 4 h, rinsed with toluene, and dried under nitrogen (Scheme 2). The wafers were allowed to cure at room temperature for 24 h.

The cured wafers were spin-coated with a solution of PEOX or PS in chloroform (10 mg/mL) using a spin-coater (P6204, Specialty Coating Systems, Inc., Indianapolis, IN) at 2000 rpm for 60 s. The films were irradiated with a medium-pressure Hg lamp (450 W, Hanovia Ltd.) for 10 min. The lamp reached its full power after ~2.5 min warm-up to an intensity of 3.5 mW/cm^2 at 18 cm from the source as measured by an OAI 306 UV power meter (Optical Associates Inc. Milpitas, CA) with a 260 nm sensor. A 280 nm optical filter was placed on the film surface during irradiation to remove the deep UV light that could cause polymer cross-linking and degradation. (The 280-nm optical filter was used in all subsequent experiments involving UV irradiation of polymers.) The films were sonicated in chloroform followed by Milli-Q water for 5 min each using a Branson 1510 sonicator (Fisher Scientific), incubated in a pH 7.4 PBS buffer at 4 °C for 2 h, rinsed with Milli-Q water, and then dried under nitrogen.

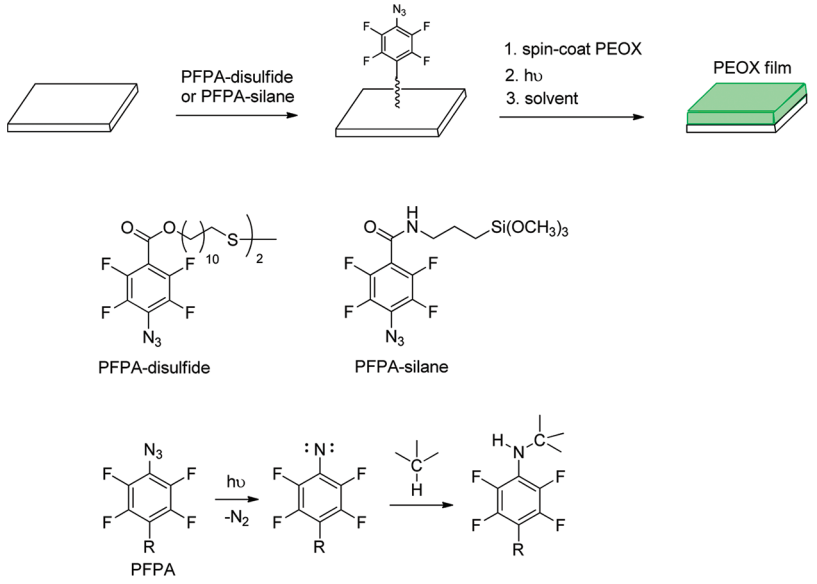
Immobilization of PEOX and PS on Gold Surfaces. Gold slides were prepared by coating piranha-cleaned glass slides with a 2 nm thick Ti followed by a 200 nm gold film in an electron beam evaporator (CrC-100 Sputtering System, Plasma Sciences Inc., Lorton, VA). The slides were cut into 1 × 1 in. pieces, cleaned in the piranha solution at room temperature for 60 s, washed in boiling water 3 times for 60 min each, and dried under a stream of nitrogen. The cleaned gold slides were soaked in a solution of PFPA-disulfide in chloroform (10 mM) for 24 h (Scheme 2). The slides were then rinsed with chloroform and dried under nitrogen.

The PFPA-functionalized gold slides were spin-coated at 2000 rpm for 60 s with a solution of PEOX or PS in chloroform (10 mg/mL). The slides were irradiated with a medium-pressure Hg lamp for 9 min, washed thoroughly in chloroform for 4 h followed by Milli-Q water for 12 h and pH 7.4 PBS buffer at 4 °C for 2 h. The slides were rinsed with Milli-Q water and dried under nitrogen.

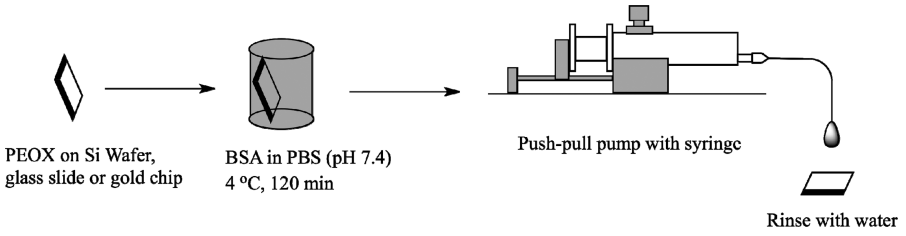
The MUTEG SAM was prepared by immersing piranha-cleaned gold slides in a solution of MUTEG in absolute ethanol (1 mM) under nitrogen for 24 h at room temperature. The slides were rinsed with ethanol and dried.

Film Thickness Measurements. Film thicknesses were measured at room temperature (~20 °C) on a Gaertner Model L116A ellipsometer

Scheme 2. Immobilization of PEOX on PFPA-Functionalized Wafer, Glass, Or Au Surface



Scheme 3. Schematic of the Protein Adsorption Experiment and the Cleaning Procedure



(Gaertner Scientific Co.) with He/Ne laser (632.8 nm, 2 mW, Melles Griot) at an incident angle of 70° in the manual mode. The real and imaginary parts of the refractive index of the silicon wafer used in the experiments were 3.870 (N_s) and −0.018 (K_s), respectively. The N_s and K_s values of the freshly cleaned gold film were measured to be 0.317 and −3.374, respectively. The following refractive indices (n_f) were used to determine the thickness of various film layers: SiO_2 1.465, PFPA-silane 1.503, PS 1.592, and PEOX 1.520. For PFPA-disulfide, a value of 1.500 was used. Eight samples were prepared for each polymer, and for each sample, nine different spots were chosen and the thicknesses were measured and averaged.

Contact Angle Measurements. Contact angles were measured on a contact angle goniometer (model 250, Ramé-Hart Instrument Co., Netcong, NJ). The advancing contact angle (θ_A) was determined by placing a drop of milli-Q water from a syringe dispenser attached to the instrument, advancing the periphery of the drop by adding water at the rate of 0.05 μL/s at a time interval of 1.0 s, and recording the contact angle as well as the diameter of the droplet. The receding contact angle (θ_R) was measured by withdrawing water from the drop at the same rate and time interval, and recording the contact angle and the diameter of the droplet. The needle was kept inside the water droplet throughout the measurements. Data were recorded and analyzed using the DROPimage Advanced v2.2 software.

Protein Adsorption and Evaluation by Ellipsometry. The BSA solution (1.0 mg/mL) was prepared in a pH 7.4 PBS buffer (0.01 M). Polymer thin films on silicon wafers or gold slides were incubated in the solution at 4 °C for 2 h, and were washed with Milli-Q water delivered by a syringe controlled by a push−pull pump (KDS-100, KD Scientific Inc., Holliston, MA) (Scheme 3). The distance between the tip of the syringe needle and the polymer film was kept at ∼2.5 cm. A total of 6.0 mL of chilled Milli-Q water (4 °C) was dripped onto the polymer film at a speed of 2 mL/min. The polymer film was then dried with nitrogen, and the thickness of adsorbed protein on each polymer film was immediately measured by ellipsometry. The N_f value, 1.45, was used for BSA on the basis of the literature data that the N_f of proteins adsorbed on surfaces was usually in the range of 1.35−1.55.[16] Nine measurements were made on each film and the data were averaged.

Fabrication of Polymer Arrays on SPR Chips. SPR chips were prepared as follows. High refractive index N-SF10 glass slides were cleaned in the piranha solution at room temperature for 60 min and washed thoroughly in boiling water three times for 60 min each. The slides were then dried with nitrogen and coated with a 2 nm thick Ti followed by a 45 nm Au film in an electron beam evaporator (SEC-600, CHA Industries, Fremont, CA) at the Microfabrication Lab, Washington Technology Center (University of Washington).

The SPR chips were cleaned with the piranha solution at room temperature for 60 s, washed in boiling water 3 times for 60 min each, and dried under a stream of nitrogen. The cleaned chips were immediately treated with PFPA-disulfide using the same procedures as those for the regular gold slides described above. PEOX arrays were generated by manually spotting solutions of PEOX in Milli-Q water onto the SPR chip using a micropipettor tip. The concentration of 30 mg/mL was used in

order to ensure the complete coverage of the polymer on the surface. The polymer-printed SPR chip was dried under the ambient condition at room temperature for 30 min followed by vacuum drying for 4 h. The chip was then irradiated for 9 min with the medium-pressure Hg lamp. The resulting sample was washed thoroughly in chloroform for 4 h followed by Milli-Q water for 12 h, and finally dried under nitrogen.

Evaluation of BSA Adsorption on PEOX Array by SPR Imaging. SPRi experiments were conducted at room temperature using a SPRimager II system (GWC Technologies, Inc.). Images were analyzed using the Digital Optics V++ Version 4 software. The angle of light incident on the prism was optimized and remained unchanged in all experiments. The polymer microarray was primed in the pH 7.4 PBS buffer until a stable baseline was reached. The BSA solution was then injected and SPR responses from all PEOX spots recorded simultaneously. The flow rate was kept at 100 μL/min. Data acquisition was conducted by selecting the area within printed spots on a microarray image, i.e., region of interest (ROI). An average of 30 images/frame was utilized and SPR signals converted to normalized percentage in reflectivity (%ΔR) following the protocol provided by GWC. All SPR images were collected using the V++ image analysis software package.

Evaluation of BSA Adsorption on Polymer Array by Fluorescence Imaging. Piranha-cleaned glass slides were soaked in a solution of PFPA-silane in toluene (12.6 mM) for 4 h, rinsed with toluene, and dried under nitrogen. The slides were allowed to cure at room temperature for 24 h. The solutions of PEOX in Milli-Q water, ethanol or chloroform (10 mg/mL) were then manually printed onto the cured glass slide using a micropipettor tip to form a polymer array. PS, spotted from a solution of PS in toluene (10 mg/mL), was also included for comparison. The polymers were covalently immobilized on the glass slide by irradiating for 10 min with the medium-pressure Hg lamp. The slide was then sonicated in chloroform followed by Milli-Q water for 5 min each to remove excess polymers, and dried under nitrogen.

A FITC-BSA solution (1.0 mg/mL) was prepared in the pH 7.4 PBS buffer (0.01 M). Glass slides containing the polymer array were incubated in the solution at 4 °C for 1 h, and rinsed with 18.0 mL chilled Milli-Q water (4 °C) through a syringe controlled by a push−pull pump at a speed of 2 mL/min (Scheme 3). The slide was dried with nitrogen, and imaged using a fluorescence array scanner (GenePix 4000B, Axon Instruments Inc., Foster City, CA) at 532 nm excitation and 575 nm emission.

Fabrication of Carbohydrate Microarrays with PEOX Film as the Antifouling Surface. Piranha-cleaned silicon wafers were soaked in a solution of GOPTS in toluene (12.6 mM) for 4 h, rinsed with toluene, and dried with nitrogen. An aqueous solution of PAAm hydrochloride (10 mg) and K_2CO_3 (25 mg) in Milli-Q water (2 mL) was mixed with a solution of *N*-hydroxysuccinimidyl 2,3,5,6-tetrafluorophenylbenzoate (PFPA-NHS) in ethanol (2 mL, 5 mg/mL), and the mixture was stirred for 24 h at room temperature to afford PFPA-functionalized PAAm, PAAm-PFPA.[49] The epoxy-functionalized wafers were then treated with the PAAm-PFPA solution prepared above at 50 °C for 5 h, sonicated in HCl (0.1 M) for 10 min to remove the unbounded PAAm-PFPA.

Aqueous solutions of different carbohydrates (10 mg/mL) were printed onto the PAAm-PFPA-functionalized wafer using a robotic printer (BioOdyssey Calligrapher miniarrayer; Bio-Rad Laboratories, Inc.). After drying under vacuum for 1 h, the wafers were spin-coated with a solution of PEOX in chloroform (10 mg/mL), and samples were irradiated with the medium-pressure Hg lamp for 9 min. The wafers were then sonicated in chloroform and Milli-Q water for 5 min each, and dried with nitrogen.

The carbohydrate microarrays were incubated in a solution of FITC-Con A (0.5 mg/mL) in HEPES buffer (pH 7.5, containing 1 mM of $CaCl_2$, and 1 mM $MnCl_2$) overnight, rinsed with HEPES buffer 3 times, and dried under nitrogen. Fluorescence images were obtained using a Genepix 4000B microarray scanner (Axon Instruments Inc., Foster City, CA) at the excitation of 532 nm. Image analysis was carried out with the Axon Genepix Pro 5.1 analysis software (Molecular Devices Corporation, Union City, CA).

Table 1. Film Thickness (d), Surface Concentration (τ), Average Distance between Polymer Chains (D), and Grafting Density (σ) for Immobilized PEOX Films of Different Molecular Weights

mol wt	d (Å)[a]	τ (μg/cm^2)	D (Å)	σ (Å^{-2})
5000	23 ± 5	0.26	18	3.2×10^{-3}
50 000	43 ± 5	0.49	41	5.9×10^{-4}
200 000	48 ± 5	0.55	78	1.6×10^{-4}
500 000	80 ± 7	0.91	95	1.1×10^{-4}

[a] Each data was the average of 8 samples. As a control experiment, a wafer without PFPA was spin-coated with PEOX 200,000. After UV irradiation and sonication in chloroform followed by Milli-Q water, the thickness was ~4 Å, which was within the error range of the instrument.

RESULTS AND DISCUSSION

Preparation and Characterization of Photoimmobilized PEOX Films. PEOX was immobilized on silicon wafers, glass slides, and gold films following a general procedure developed previously in our laboratory. Briefly, silicon wafers or glass slides were treated with PFPA-silane,[35,37] and gold films were treated with PFPA-disulfide[47] to introduce PFPA on the surface (Scheme 2). PEOX was then spin-coated on the PFPA-functionalized surfaces followed by photolysis when PFPA was converted to the highly reactive singlet perfluorophenyl nitrene to form covalent bonds with the polymer via a CH insertion reaction (Scheme 2). After the excess polymer was removed by sonication in a solvent such as chloroform, PEOX thin films were obtained.

PEOX of various molecular weights (5000, 50 000, 200 000, and 500 000) were used in this study. The thickness of immobilized PEOX films was measured by ellipsometry, and results show that the film thickness increased with the molecular weight of the polymer (Table 1). This is expected considering that the covalent bond formation occurs at the interface between the surface PFPA and the coated PEOX (Scheme 2). Only a monolayer of polymer remained on the surface and the thickness of the film was proportional to the radius of gyration of the polymer.[35]

The surface concentration, the average distance between immobilized PEOX chains, and the grafting density of the PEOX films were calculated, and results are shown in Table 1. The surface concentration, τ, defined as the mass of the immobilized polymer per unit area, can be calculated by applying eq 1,[50] where d is the thickness of the PEOX film, and ρ_{dry} is the density of the film. Here, the bulk density of PEOX, 1.14 g/cm^3, was used as an estimation.[51]

$$\tau(\mu g/cm^2) = d\rho_{dry} \quad (1)$$

The average distance between the immobilized polymer coils, D, is computed from eq 2, where M is the molecular weight of the polymer and N_A is the Avogadro's number. The equation is derived by assuming the unit surface area of a single polymer

Table 2. Static Contact Angle, θ_A, θ_R, and Hysteresis of the Immobilized PEOX Films

mol wt	static contact angle (deg)[a]	θ_A (deg) [a]	θ_R (deg)[a]	hysteresis (deg)[b]
5000	43.3 ± 1.0	53.5 ± 2.0	26.0 ± 1.0	27.5 ± 1.3
50 000	39.6 ± 0.7	45.1 ± 0.7	23.9 ± 0.9	21.2 ± 1.3
200 000	39.4 ± 0.5	44.8 ± 1.1	23.9 ± 1.3	21.9 ± 2.0
500 000	41.5 ± 0.5	46.4 ± 1.0	23.2 ± 1.1	23.3 ± 1.2

[a] Each data was the average of 5 samples. [b] Hysteresis, $\theta_A - \theta_R$, was computed from each of the 5 samples individually, and the results were averaged.

chain as D^2. Therefore, the weight of a single polymer is $D^2\rho_{dry}d$, which also equals to M/N_A.

$$D^2\rho_{dry}d = M/N_A \quad (2)$$

The grafting density, σ, defined as the number of molecules per unit area, can be calculated according to eq 3.[52,53]

$$\sigma = (\rho_{dry}dN_A)/M = 1/D^2 \quad (3)$$

The surface concentration, τ, increased with the molecular weight, a result that is consistent with the thickness data (Table 1). The distance between immobilized polymer chains, D, followed the same trend that it increased with the molecular weight, indicating that the longer chains occupy more space than the shorter ones. The number of polymer chains per unit area, i.e., the grafting density (σ), was inversely related to the molecular weight. These data compare well with those of end-grafted PEOX films reported by others. In the case of Rehfeldt and co-workers where PEOX brushes were immobilized on silicon wafers via a terminal trimethoxysilane group, the film thicknesses were reported to be 24.4−39.7 Å for PEOX 3,000.[34] Therefore, the D and σ values, calculated from eqs 2 and 3, are 10.5−13 Å and 5.6×10^{-3} to 9.1×10^{-3} Å^{-2}, respectively. For PEOX 5,000 films fabricated by our photoimmobilization method, the grafting density was 3.2×10^{-3} Å^{-2}, which is on the same order of magnitude as those PEOX brushes.

Dynamic contact angle measurements were carried out to monitor the PEOX immobilization process. The static water contact angle of the PFPA-functionalized silicon wafer was around 78°, which decreased to 39−43° after the PEOX film was immobilized (Table 2). The advancing and receding contact angles were then measured (see Figure S1 in the Supporting Information for the advancing and receding contact angle measurement graphs), and hysteresis computed for each PEOX film. The results were similar for PEOX of higher molecular weights, however, for the PEOX 5000 films, a slightly larger hysteresis was obtained (Table 2). The contact angle hysteresis measures the adhesion of the liquid droplet, i.e., water in this case, to the surface. The value is influenced by the surface roughness, chemical heterogeneity, and the extent of interactions between the water droplet and the surface constituents.[54] An increase in contact angle hysteresis may imply a surface that is more heterogeneous, rougher or less stable. The PEOX 5000 film was considerably thinner, and is of lower surface concentration as compared to the higher molecular weight PEOX films (Table 1).

Evaluation of Protein Adsorption by Fluorescence Imaging. The protein-resistant property of the photoimmobilized PEOX films was next evaluated. Similar to PEG, PEOX is soluble in both water and polar organic solvents, which is a significant advantage with regard to its practice uses. Experiments were carried out to test the effect of the solvent on the protein absorption of the resulting films. PEOX 500 000 was dissolved in chloroform, ethanol or Milli-Q water at 10 mg/mL, and the solutions were spotted on the PFPA-functionalized glass slide. In addition to PEOX, PS, a well-known protein fouling polymer, was also spotted on the same slide as a reference. The sample was irradiated to attach the printed polymers, and excess polymers were removed by sonication in chloroform. The glass slide containing the polymer array was then treated with FITC-BSA in pH 7.4 PBS buffer, rinsed with the fresh buffer under controlled conditions using a syringe pump (Scheme 2), and analyzed using a microarray scanner. The PS spots showed the brightest fluorescence as expected (Figure 1). On the other hand, the fluorescence intensities on the PEOX spots were significantly lower. Furthermore, PEOX films spotted from the chloroform solution showed slightly higher fluorescence intensity than the films made from the ethanol or the aqueous solution. It is generally accepted that proteins preferably adsorb on hydrophobic over hydrophilic surfaces as their interactions with hydrophobic surfaces are more energetically favorable.[55,56] In this case, the PEOX films prepared from the chloroform solution may have more hydrophobic domains exposed on the surface causing higher protein adsorption.

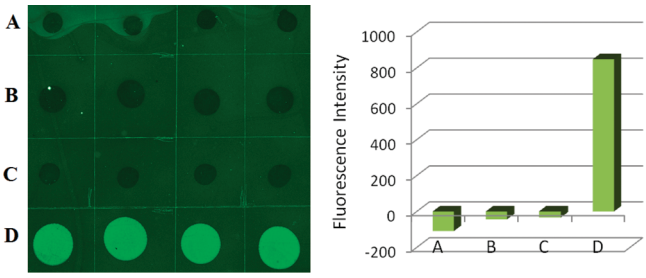


Figure 1. Fluorescence image (left) and intensity (right) of polymer array after incubating with FITC-BSA. The array consists of photoimmobilized PEOX 500 000 spotted from (A) Milli-Q water, (B) ethanol, (C) chloroform, and (D) PS 280 000 from toluene.

To test this hypothesis, we carried out contact angle measurements to compare the hydrophobicity of the films. A solution of PEOX 500 000 (10 mg/mL) in chloroform, ethanol, or Milli-Q water was dropped on the PFPA-modified wafers, followed by UV-irradiation and sonication in chloroform and Milli-Q water, and then dried under nitrogen. The static water contact angles of the films prepared from chloroform, ethanol and Milli-Q water were 43.0 ± 0.4, 40.5 ± 0.7, and 40.3 ± 1.2°, respectively, indicating that the film prepared from the chloroform solution was slightly more hydrophobic than the films prepared from the ethanol or the aqueous solution. The advancing contact angle followed the same trend that the values were slightly higher for films prepared from the chloroform solution than those from the aqueous solution (Table 3). A smaller contact angle hysteresis was obtained for the PEOX film prepared from the chloroform solution, indicating a smoother or more homogeneous surface (Table 3).[54]

Evaluation of Protein Adsorption by Ellipsometry. The protein-resistant property of the photoimmobilized PEOX films was subsequently studied by subjecting the films to BSA treatment, and the amounts of BSA adsorbed on the films were measured by ellipsometry. The films were prepared from the chloroform solutions since the films were more smooth and

Table 3. Static Contact Angle, θ_A, θ_R, and Hysteresis of PEOX 500 000 Films Prepared from the Chloroform and the Aqueous Solutions

solvent	static contact angle (deg)[a]	θ_A (deg)[b]	θ_R (deg)[b]	hysteresis (deg)[c]
chloroform	43.0 ± 0.4	45.8 ± 0.6	21.2 ± 0.5	24.6 ± 0.8
Milli-Q water	40.3 ± 1.2	44.2 ± 2.7	18.0 ± 1.8	26.3 ± 1.7

[a] Each value was the average of 8 samples. [b] θ_A and θ_R values were computed from measurements of 3 samples each. (see Figure S2, Supporting Information, for the advancing and receding contact angle measurement graphs) [c] Hysteresis was computed from each of the 3 samples, and the results were averaged.

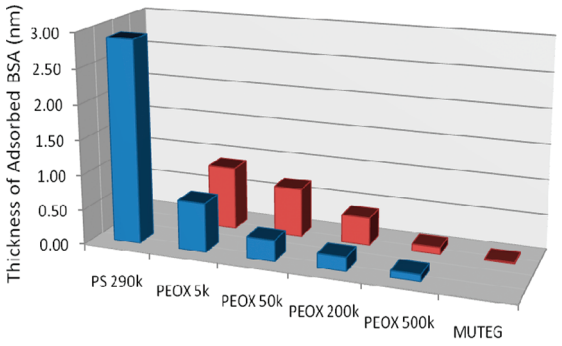


Figure 2. Thicknesses of adsorbed BSA on PS, PEOX, and MUTEG SAM immobilized on silicon wafer (front row) and gold slide (back row). Each value represents the average of 18−36 measurements (2−4 samples, and 9 measurements at different spots on each sample). The error bars were omitted for clarity.

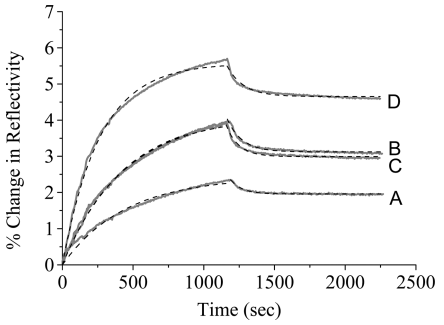


Figure 3. SPR sensorgrams monitoring the adsorption of BSA (0.75 mg/mL in pH 7.4 PBS buffer) on immobilized films of (A) PEOX 500 000, (B) PEOX 50 000, (C) PEOX 200 000, and (D) PEOX 5000. Both experimental data (solid lines) and fitted curves (dotted lines) are shown. The BSA solution was injected at 0 s, and the fresh PBS buffer was introduced at 1200 s. The polymer array consisted of the above 4 PEOX samples (see Figure S3, Supporting Information for the array layout and the SPRi image of the array). For clarity, only one sensorgram is shown for each polymer.

Table 4. Association Rate Constant, k_a, Dissociation Rate Constant, k_d, and the Adsorption Constant, K, of BSA Adsorption on PEOX Films Obtained by SPRi

mol wt	k_a ($\times 10^2$ s^{-1} M^{-1})	k_d ($\times 10^{-2}$ s^{-1})	K ($\times 10^4$ M^{-1})
500 000	0.93	1.40	0.66
200 000	1.80	1.23	1.46
50 000	1.63	1.19	1.37
5000	1.33	0.81	1.64

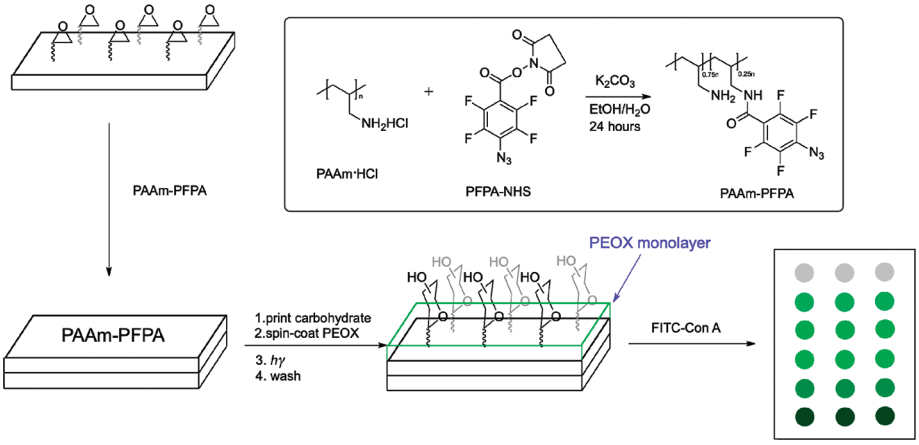
homogeneous than those prepared from the aqueous solutions (Table 3). The fouling polymer, PS, and a protein-resistant surface, MUTEG SAM on gold,[16] were used as the references in this study. As expected, all PEOX films showed significantly lower BSA adsorption in comparison to PS (Figure 2). In addition, the amount of adsorbed BSA decreased with increasing molecular weight of PEOX, and PEOX 500,000 showed the least amount of BSA adsorption. The polymer surface concentration is an important parameter affecting the protein-resistant property of nonfouling polymers such as PEG. It is generally believed that nonfouling polymers act by creating a barrier for proteins through entropic repulsion, osmotic repulsion and excluded volume effects.[13,57,58] The low protein adsorption observed on PEOX 500 000 is likely due to the high polymer surface concentration, which increased with the molecular weight of PEOX (Table 1).

Evaluation of Protein Adsorption by SPRi. The protein-resistant property of the immobilized PEOX films was further evaluated using SPRi. SPRi is a label-free and real-time sensing technique, and it allows the simultaneous measurements of multiple interactions in an array format.[59−65] Kinetic analyses are also possible, which afford the rate and equilibrium constants of the interactions. In this study, a polymer array consisting of PEOX 5000, 50 000, 200 000, and 500 000 was prepared on a PFPA-functionalized SPR sensor chip. All PEOX were spotted from their aqueous solutions and were subsequently attached to the SPR chip by photoimmobilization. The kinetics study is conducted in two phases. The first is the association phase where the BSA solution was introduced to the flow cell and the interactions between BSA and the polymer occurred. In the second dissociation phase, the fresh buffer was introduced such that the adsorbed BSA would desorb from the polymer surface. This adsorption−desorption process for each polymer spot was monitored simultaneously in real time by SPRi; typical sensorgrams are shown in Figure 3. It can be seen that the amount of BSA adsorbed was the lowest on PEOX 500 000, and the lowest on PEOX 5000. This result is consistent with the ellipsometry results shown in Figure 2.

Titration experiments were then conducted where the concentration of BSA was varied and the SPR responses were recorded. The data obtained were analyzed assuming a first-order kinetics. Note that this is a simplified model for the complex protein adsorption process that includes protein−surface interactions, protein conformation changes, surface diffusion, and so forth. The observed association rate constants (k_{obs}) were determined from a series of BSA titration experiments by fitting the corresponding SPR curves (see the Supporting Information for details). The k_{obs} values were then plotted against the BSA concentration. A linear curve was obtained and the association rate (k_a) was then determined from the slope of the line (see the Figure S4, Supporting Information).

The dissociation rate constant (k_d) was determined by fitting the SPR curves of the BSA dissociation phase, also assuming a first-order kinetic process (see the Supporting Information for details). The adsorption constant ($K = k_a/k_d$) was then computed, and the values together with k_a and k_d for various molecular weight PEOX are shown in Table 4. In general, a good protein-resistant surface would have a smaller k_a, i.e., slower

Scheme 4. Fabrication of Carbohydrate Microarray with PEOX as Anti-fouling Coating



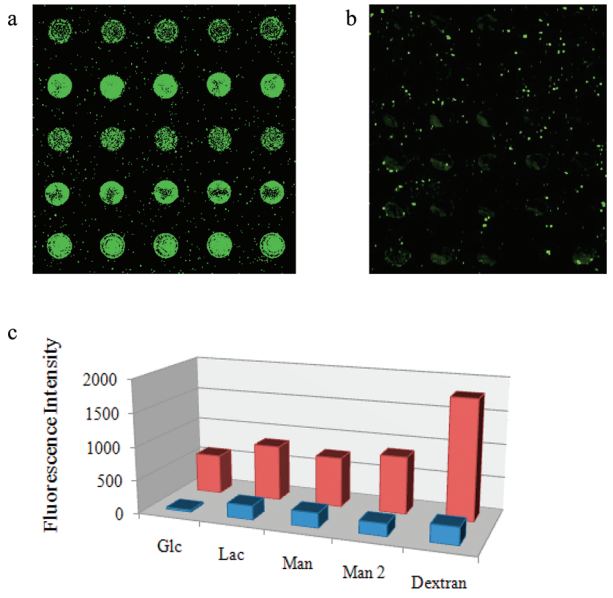


Figure 4. Fluorescence image of carbohydrate microarray probed by FITC-Con A (a) with or (b) without PEOX. From top to bottom in a and b, the printed carbohydrates are Glc, Man, Lac, Man2, and dextran, respectively. (c) Fluorescence intensities of carbohydrate microarray in (a) (back row) and (b) (front row). Each value represents an average of 10 measurements; the error bars are omitted for clarity.

adsorption rate, and a larger k_d, i.e., faster desorption rate. Results in Table 4 show that PEOX 500 000 gave the smallest k_a and the largest k_d values. These data are again in agreement with the results obtained from the fluorescence imaging and ellipsometry studies, demonstrating that the antifouling property of PEOX films is molecular weight dependent with the PEOX 500 000 giving the best protein-resistant surface.

PEOX as Anti-Fouling Coating in Carbohydrate Microarrays. The protein-resistant property of PEOX was utilized in the fabrication of carbohydrate microarrays where PEOX films were incorporated as antifouling coatings (Scheme 4). Aqueous solutions of 5 different carbohydrates, Man, Glc, Lac, Man2, and dextran, were spotted on PAAm-PFPA-functionalized wafers[49] using a robotic printer followed by spin coating a solution of PEOX in chloroform. Chloroform was chosen as the solvent to avoid disturbing the spotted carbohydrates which are insoluble in organic solvents. The sample was then irradiated to covalently attach both carbohydrates and PEOX to the wafer via the covalent bond formation with the surface PFPA. After removing the excess PEOX and carbohydrates by washing the wafer with chloroform and water, the resulting sample was treated with FITC-Con A. Con A is a plant lectin that exhibits specific affinity for Man and Glc. As expected, the spots of Man- and Glc-containing carbohydrates showed high fluorescence intensities (Figure 4a). A second microarray was then fabricated in the same manner except that the PEOX was omitted. In this case, very weak fluorescence was observed (Figure 4b), and the intensities of the spots on the array (front row, Figure 4c) were significantly lower than those on the array where PEOX was used (back row, Figure 4c). The lower signal intensities can be attributed to the higher background noises resulting from the nonspecific adsorption of FITC-Con A. In Figure 4a, the PEOX coating in the microarray reduced the nonspecific adsorption of FITC-Con A. The background noises therefore decreased and the microarray signals were drastically enhanced as a result.

CONCLUSIONS

The light-activated immobilization of PEOX proved to be a fast, efficient, and versatile method to generate covalently bound PEOX films on silicon wafers, gold films, and glass slides. The method applies to PEOX of varying molecular weights and the samples can be readily obtained from commercial sources without the need for synthesis or chemical derivatization. The grafting density of the photoimmobilized films was comparable to those reported for other end-grafted PEOX films. The antifouling property of the films was evaluated by fluorescence imaging, ellipsometry, and SPRi. Results show that the protein resistant property of PEOX was molecular weight dependent, and PEOX 500 000 was the best in resisting BAS adsorption. Similar to PEG, PEOX has excellent solubility in both organic and aqueous solutions, which is a significant advantage for its practical uses. Compared with PEG that is difficult to produce smooth films due to its high crystallinity, PEOX can be easily fabricated into higher quality films by spin-coating. The method developed has been successfully integrated with the microarray

fabrication process where covalently immobilized PEOX films significantly reduced the background noises and greatly enhanced the signal sensitivity.

■ ASSOCIATED CONTENT

Supporting Information. Contact angle and drop diameter graphs, polymer microarray and the corresponding SPRi image, adsorption kinetics analysis by SPR. This material is available free of charge via the Internet at http://pubs.acs.org/.

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