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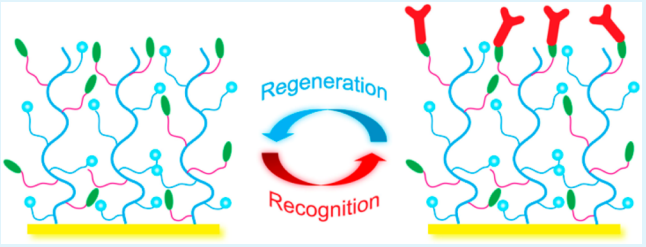
Specificity and Regenerability of Short Peptide Ligands Supported on Polymer Layers for Immunoglobulin G Binding and Detection

Yanxia Zhang,*[,†] Nafisa Islam,[‡] Ruben G. Carbonell,[‡] and Orlando J. Rojas*[,†,‡]

[†]Department of Forest Biomaterials, North Carolina State University, Raleigh, North Carolina 27695, United States

[‡]Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, North Carolina 27695, United States

ABSTRACT: We demonstrate the specificity, regenerability, and excellent storage stability of short peptide-based systems for detection of immunoglobulin G (IgG). The bioactive component consisted of acetylated-HWRGWVA (Ac-HWRGWVA), a peptide with high IgG binding affinity, which was immobilized onto copolymer matrixes of poly(2-aminoethyl methacrylate hydrochloride-*co*-2-hydroxyethyl methacrylate) (poly(AMA-*co*-HEMA)). Surface plasmon resonance (SPR) and quartz crystal microgravimetry (QCM) were utilized with other complementary techniques to systematically investigate interfacial activities, mainly IgG binding performance as a function of the graft density and degree of polymerization of the poly(AMA-*co*-HEMA) support layer. Results from sodium dodecyl sulfate polyacrylamide gel electrophoresis and fluorescence microscopy indicate that the bioactive system is highly specific to IgG and resistant to nonspecific interactions when tested in mixed protein solutions.



KEYWORDS: *short peptide ligands, immunoglobulin G detection, surface plasmon resonance, quartz crystal microgravimetry, regenerability, storage stability*

INTRODUCTION

Immunoglobulins are major components of the immune system in response to immunogens. Immunoglobulin G (IgG) is the most versatile immunoglobulin because it is capable of carrying out all of the functions of immunoglobulin molecules and thus has been widely used in biotechnology and medicine.[1−4] Many related applications demand biosensors for the detection of IgG; they not only can help to control and optimize IgG bioseparation and purification processes but also can serve as indicators of many autoimmune diseases related to IgG, which is present in concentrations ranging from 6.6 to 14.5 mg/mL in normal human plasma.[5−8] To date, the most widely used IgG ligands are proteins A and G. Despite their relative high affinities for IgG (affinity constant of 10^7−10^8 M^{-1}),[9−11] these proteins present drawbacks such as their high cost and low stability during washing and regeneration.[12−14] From this perspective, many attempts have been made to develop new affinity ligands for IgG detection.[2,15−21] Among them, small synthetic peptide ligands have received considerable attention, since they are cost-effective, display high stability, and are robust and easy to store. From combinatorial solid-phase hexamer peptide libraries, Carbonell et al. have identified HWRGWV as a candidate short peptide to selectively bind the Fc region of IgG. It was found that chromatography resins based on acetylated-HWRGWV (Ac-HWRGWV) were able to purify IgG from complete mammalian cell culture medium (cMEM) and commercial Chinese hamster ovary (CHO) cell culture supernatant with comparable efficiency as protein A/G but at a much reduced cost.[14,22−24] Overall, these results highlighted HWRGWV as an efficient ligand for IgG detection.

An ideal biosensor having optimal performance must (a) provide high binding capacities for target molecules; (b) reduce the interference from nonspecific interactions; (c) prevent denaturation of the immobilized ligands; and (d) allow convenient and efficient immobilization, ideally reversible to allow regeneration.[25−30] Hydrophilic polymers can provide a nonfouling background and are widely adopted as a sensor matrix or spacer to integrate affinity ligands.[31,32] Recently, we developed a bioactive system consisting of Ac-HWRGWVA supported on a poly(2-aminoethyl methacrylate hydrochloride-*co*-2-hydroxyethyl methacrylate) (poly(AMA-*co*-HEMA)) matrix.[33] This peptide-based system was shown to be capable of detecting IgG from buffer solutions at concentrations as low as 0.05 mg/mL while maintaining nonspecific protein resistance. Especially, the affinity constant for IgG binding was determined to be 4.9×10^5 M^{-1}, which is lower than that of protein A but more appropriate for recovery of IgG, thus providing a better potential for regeneration under milder elution conditions. However, important issues such as the influence of the supporting polymer matrix on the binding capability and specificity of the system in the presence of interfering proteins are unknown and an evaluation of the reproducibility, repeatability, as well as storage stability of the peptide systems

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was lacking. Therefore, this study addresses these issues by a systematic investigation of the structures and properties of poly(AMA-*co*-HEMA)−peptide systems.

MATERIALS AND METHODS

Materials. 2-Hydroxyethyl methacrylate (HEMA), 2-aminoethyl methacrylate hydrochloride (AMA), 2,2′-bipyridine, copper(II) bromide ($CuBr_2$), diluents thiol (11-mercaptoundecyl) tri(ethylene glycol), *N*,*N*-dimethylformamide (DMF, anhydrous), *N*,*N*-diisopropylethylamine (DIPEA), *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU), fluorescein isothiocyanate-labeled IgG from human serum (FITC−IgG), and albumin from bovine serum (BSA) were purchased from Sigma-Aldrich (Milwaukee, WI) and used as received. Initiator thiol (ω-mercaptoundecyl bromoisobutyrate) was purchased from Prochimia (Sopot, Poland). Short peptide, acetylated-HWRGWVA (Ac-HWRGWVA, 95.1%), was purchased from GenScript (Piscataway, NJ). Human IgG (>97%) was obtained as a lyophilized powder from Equitech-Bio (Kerrville, TX). QCM and SPR gold chips were purchased from Q-Sense (Göteborg, Sweden) and BioNavis (Tampere, Finland), respectively.

Poly(AMA-*co*-HEMA)−Peptide System. *Preparation of Mixed SAM Functionalized Surfaces.* Gold chips (QCM and SPR chips) were cleaned in "piranha" solution (H_2SO_4:H_2O_2 = 7:3 (v/v)) (***Caution: piranha solution reacts violently with organic materials and should be handled carefully***) for 5 min at room temperature to remove the organic residues. The surfaces were then rinsed with abundant Milli-Q water, dried under a nitrogen flow, and exposed to UV radiation for 30 min just before use.

The freshly prepared solid supports were immersed into an anhydrous ethanol solution containing initiator thiol and diluent thiol with a desired solution ratio (keeping the total concentration at 1 mM) for 15 h at room temperature. This allowed the formation of binary, mixed self-assembled monolayers (SAMs) on the surfaces. The solution ratio of initiator, χ_I^{Sol}, was defined as $\chi_I^{Sol} = M_{initiator}/(M_{initiator} + M_{diluent}) \times 100\%$, where M is molar units. The mixed SAM-functionalized surfaces were thoroughly rinsed with ethanol and then dried under a nitrogen flow.

Preparation of Poly(AMA-co-HEMA) Surfaces. The random copolymer, poly(AMA-*co*-HEMA), was grafted from the mixed SAM-functionalized surfaces via surface initiated activators regenerated by electron transfer atom transfer radical polymerization (ARGET-ATRP). The reaction solution with a AMA:HEMA mole ratio of 20:80 was prepared by dissolving AMA (0.36 g, 2.2 mmol), HEMA (1.15 g, 8.8 mmol), bipyridine (7.7 mg, 49 μmol), $CuBr_2$ (1.6 mg, 7 μmol), and ascorbic acid (17.3 mg, 98 μmol) into a mixture of methanol (7 mL) and water (7 mL). The surfaces were immersed into the reaction solution for given time periods at room temperature. Then, the surfaces were removed from the solution, rinsed with abundant Milli-Q water and anhydrous methanol to remove unreacted monomers and physically adsorbed polymers, and dried under a nitrogen flow. As a result, supporting layers of poly(AMA-*co*-HEMA) with different grafting densities and thicknesses were obtained.

Preparation of Poly(AMA-co-HEMA)−Peptide Surfaces. Short peptides, Ac-HWRGWVA, were covalently immobilized onto the poly(AMA-*co*-HEMA) layer via amide bonds formed between the carboxyl groups of the peptide and the amine groups of AMA segments. Briefly, poly(AMA-*co*-HEMA) surfaces were immersed into an anhydrous DMF solution containing Ac-HWRGWVA (5 mg/mL) and HATU (3.8 mg/mL), followed by addition of DIPEA (2.58 μL/mL). The reaction continued under an atmosphere of nitrogen for 12 h at room temperature. Finally, the surfaces were rinsed with DMF and Milli-Q water thoroughly to remove the impurities, and dried under a nitrogen flow to achieve the poly(AMA-*co*-HEMA)−peptide surfaces.

Surface Characterizations. *Ellipsometry.* The thickness of the grafted polymer layer was measured by a spectroscopic ellipsometer (model M-2000 V, J. A. Woollam Co., Inc.) at an angle of 70° and wavelengths from 400 to 800 nm. Ellipsometric data were fitted using a Cauchy layer model with fixed (A_n, B_n) values of (1.46, 0.01). The thickness for each sample was measured at three different locations and was reported as the average.

X-ray Photoelectron Spectroscopy (XPS). The element composition of surfaces was determined by an X-ray photoelectron spectrometer (SPECS, Germany) with a monochromatic Mg Kα X-ray source (1253.6 eV) under 10^{-8}−10^{-9} Torr pressure. Measurements were made at two different takeoff angles, 30 and 90°. All binding energies were referenced to the main hydrocarbon C1s peak, designated at 285 eV.

IgG Detection and Nonspecific Protein Adsorption. Protein adsorption on poly(AMA-*co*-HEMA)−peptide surfaces was examined by several techniques. In our previous work, we prepared affinity chromatography systems with HWRGWV as a ligand to purify IgG from IgG spiked mammalian cell culture medium (cMEM), which contained 10% fetal calf serum and 5% tryptose phosphate broth.[12] We found that BSA was the main contaminant in IgG purification and had a much higher binding affinity with the peptide than any other proteins in cMEM. Therefore, in this work, BSA was used as a representative nonspecific protein in the detection of IgG target molecule. They were dissolved at different concentrations in phosphate-buffered saline (PBS, 150 mM, pH 7.4) with addition of 1 M NaCl (denoted as PBSS for clarity). The addition of NaCl can greatly decrease nonspecific electrostatic binding of BSA and increase the purity and yield of IgG in cMEM.[14]

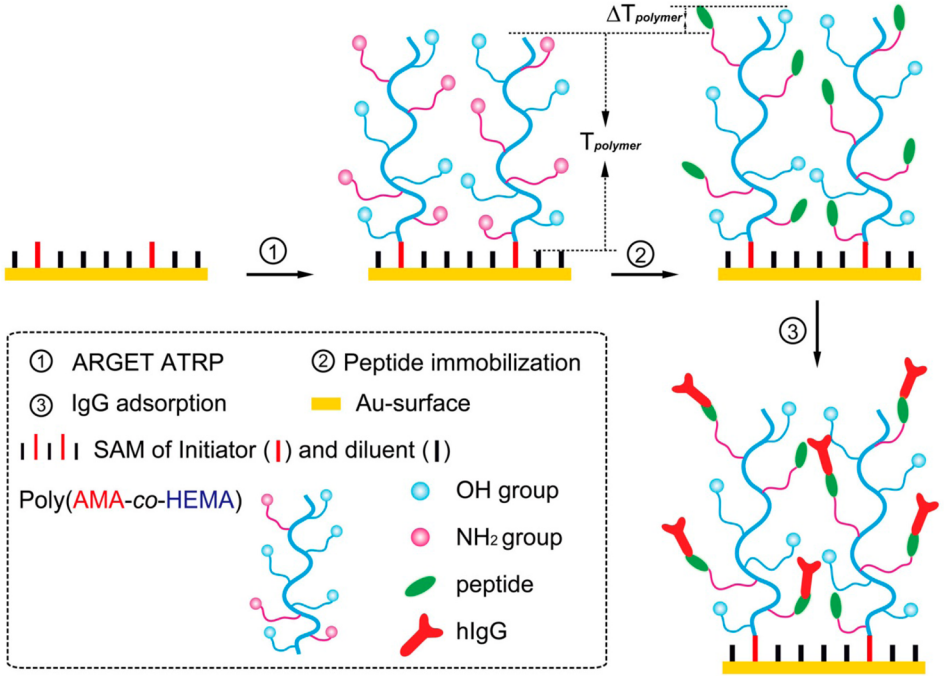
Quartz Crystal Microgravimetry (QCM). QCM measurements were performed using a Q-Sense E4 system (Gothenburg, Sweden) at 25 °C. The modified QCM sensors were mounted, and PBSS running buffer was pumped through the system by a peristaltic pump (model ISM 935, Ismatec, Switzerland) until a stable baseline was recorded. Protein adsorption measurements were conducted by injecting the respective protein solution in the QCM chamber at a flow rate of 50 μL/min. At the end of the experiment, the protein running solution was replaced by injection of PBSS. Time-resolved changes in the shift of resonant frequency were recorded and analyzed using the "solidified liquid layer" model to quantify the surface excess.[34]

Surface Plasmon Resonance (SPR). Protein adsorption on poly(AMA-*co*-HEMA)−peptide surfaces was also measured using a SPR (KSV, Helsinki, Finland) operated in a flow-through mode with a flow rate of 10 μL/min at 25 °C. PBSS running buffer was pumped to the poly(AMA-*co*-HEMA)−peptide surface to reach a stable baseline, and then protein solution (1 mg/mL) was injected into the system. Finally, PBSS was passed through the surface until reaching another baseline. The changes of SPR signals were used to quantitatively determine the level of protein adsorption.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE). The selectivity of the poly(AMA-*co*-HEMA)−peptide system to IgG in the presence of interfering BSA was investigated by SDS-PAGE. Instead of gold chips, glass coverslips (Fisherbrand, 22 × 22 mm, Fisher Scientific) were used as substrates to ensure the identical properties on both sides of the solid support. The cleaned coverslips were immersed in anhydrous toluene containing 1% (v/v) ATRP initiator terminated silane ((3-trimethoxysilyl) propyl 2-bromo-2-methylpropionate (Gelest, Inc., Morrisville, PA)) for 24 h at room temperature to generate brominated surfaces. Poly(AMA-*co*-HEMA)−peptide modified glass surfaces were prepared using the same protocol described for gold chips. These surfaces were incubated in IgG (1 mg/mL) containing BSA (1 mg/mL) in PBSS buffer for 2 h at room temperature. Following adsorption, the surfaces were rinsed with PBSS for three times and wicked onto filter paper. The adsorbed proteins were eluted from the surfaces by incubating the samples in 2% aqueous sodium dodecyl sulfate (SDS) aqueous solution for 2 h at room temperature. To maximize the eluted protein concentration, a very small amount of SDS was used (100 μL) by breaking the glass surfaces into very small pieces in a 2.5 mL centrifuge tube.

The eluted proteins were analyzed by reduced SDS-PAGE using NuPAGE Novex 12% Bis-Tris gels (Invitrogen, Carlsbad, CA, USA) on an XCell SureLock Mini-Cell system from Invitrogen. The gels were stained using the colloidal blue staining kit (Invitrogen). Sample preparation was done by adding 10 μL of NuPAGE LDS buffer and 4 μL of NuPAGE reducing agent to 40 μL of sample solution, and the

Scheme 1. Schematic Illustration of the Preparation of Poly(AMA-*co*-HEMA)−Peptide Surfaces for IgG Detection



resulting mixture was boiled for 10 min before it was loaded onto the gel.

Fluorescence Microscopy. The adsorption of FITC−IgG on the poly(AMA-*co*-HEMA)−peptide surface with and without interfering BSA was evaluated using an Olympus BX-61 optical microscope operated under transmitted and fluorescence mode. The modified surfaces were equilibrated in PBSS for 12 h prior to incubation in 0.2 mg/mL FITC−IgG with and without BSA (0.2 mg/mL) in PBSS for 2 h at room temperature. Before imaging, the surfaces were rinsed three times with PBSS and Milli-Q water and then dried under a gentle flow of nitrogen. All images used for comparison of fluorescence intensities were obtained using identical exposure times, image contrast, and brightness settings. For each sample, six images from random areas across the sample surface were captured and analyzed to obtain the average fluorescence intensity.

Reproducibility and Regenerability of the Peptide-Based System. The reproducibility and regenerability of the peptide-based system were investigated using the QCM technique. Several different eluting buffers including 6 M guanidine−HCl, 2% SDS, 0.2 M Glycine−HCl buffer, and a mixed solution of 0.1 M NaOH with 10% of acetonitrile were used.

First, PBSS running buffer was pumped to the poly(AMA-*co*-HEMA)−peptide surface at a speed of 50 μL/min to reach a stable baseline and then 0.5 mg/mL IgG solution was injected into the system for 20 min to achieve peptide−IgG binding; the modified surfaces were rinsed with PBSS thoroughly to remove nonspecific IgG adsorbed until reaching another baseline. Then, different elution buffers were pumped in the system for another 20 min, and after elution, the surfaces were washed in PBSS solution for re-equilibration. To test the activity of the peptide system after the treatment of elution buffer, another cycle of IgG adsorption was applied, in a similar manner as that described above.

Storage Stability of the Peptide Sensing System. The storage stability of the peptide system was investigated by using QCM. The as-prepared poly(AMA-*co*-HEMA)−peptide surfaces were stored in air or in PBS at 4 °C. After a desired time period (from 1 to 20 days), the sensors were washed three times for 10 min with Milli-Q water and dried under a nitrogen flow.

RESULTS AND DISCUSSION

Surface Preparation. A series of poly(AMA-*co*-HEMA)−peptide surfaces were prepared, as illustrated in Scheme 1. First, mixed SAMs containing initiator thiol and dilute thiol were formed on gold surfaces. Here, (11-mercaptoundecyl) tri(ethylene glycol) was used as a diluent to adjust the surface density of initiator and as an additional barrier to nonspecific protein adsorption.[31,32,35,36] Then, ARGET-ATRP of AMA and HEMA was conducted from the mixed SAMs. The layer thickness of the copolymer was controlled by adjusting the polymerization time.[33] The HEMA segments provide nonspecific protein resistance, while the AMA segments in the copolymer endow the system with peptide immobilizing capability.[20,26,37−39] We found that the copolymer with the feed mole ratio of AMA:HEMA of 20:80 exhibited an optimal balance between specific recognition of IgG binding and resistance to nonspecific interactions;[33] therefore, this ratio was used in the present study. Finally, the peptide was covalently immobilized onto the polymer matrix via amide chemistry using HATU as a coupling agent. The resultant poly(AMA-*co*-HEMA)−peptide surfaces and their functionality were evaluated.

Effects of Polymer Matrix Density and Thickness on IgG Binding. It is widely known that thickness and graft density are two crucial parameters influencing the properties of surface-tethered polymer brushes; it is thus expected that they also affect sensor performance when they are used as matrixes for (bio)active components.[32,40−42] In this study, we systematically investigated the influence of these factors on the binding capability of IgG using SPR and QCM. SPR signals are directly related to the amount of protein molecules present in the adsorbed layer (*dry mass*), while the QCM frequency shifts are related to the total mass including hydration or coupled water associated with the adsorbed proteins (*wet mass*).[43,44]

First, we investigated the influence of the thickness of poly(AMA-*co*-HEMA) ($T_{polymer}$) on the extent of protein adsorption. A series of surfaces with a constant, 100% χ_I^{Sol}

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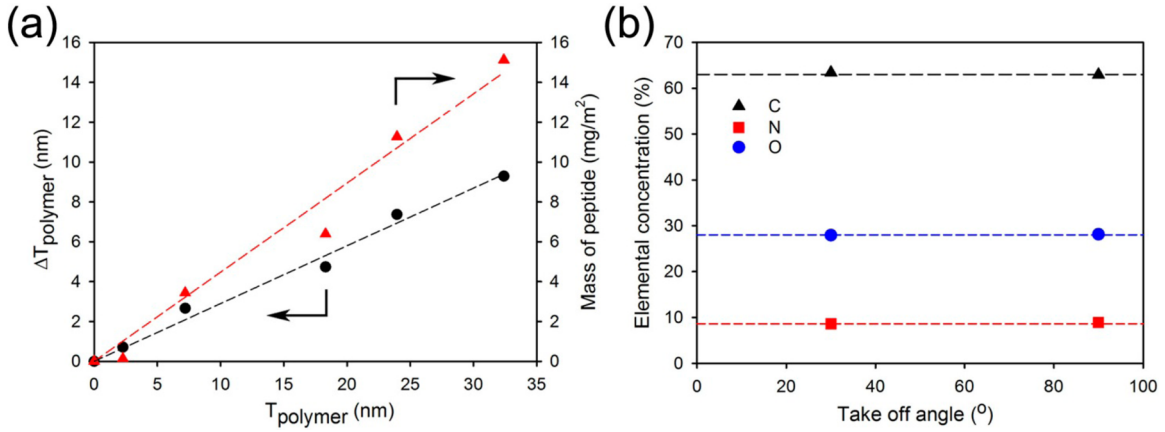

Figure 1. Short peptide ligands were immobilized evenly within poly(AMA-*co*-HEMA) layers: (a) Linear increase in thickness and mass upon peptide immobilization on poly(AMA-*co*-HEMA) supports of different initial thicknesses. (b) Angle-resolved XPS for atomic surface concentration (% C, N, O) in poly(AMA-*co*-HEMA)−peptide (takeoff angles of 30 and 90° are shown).

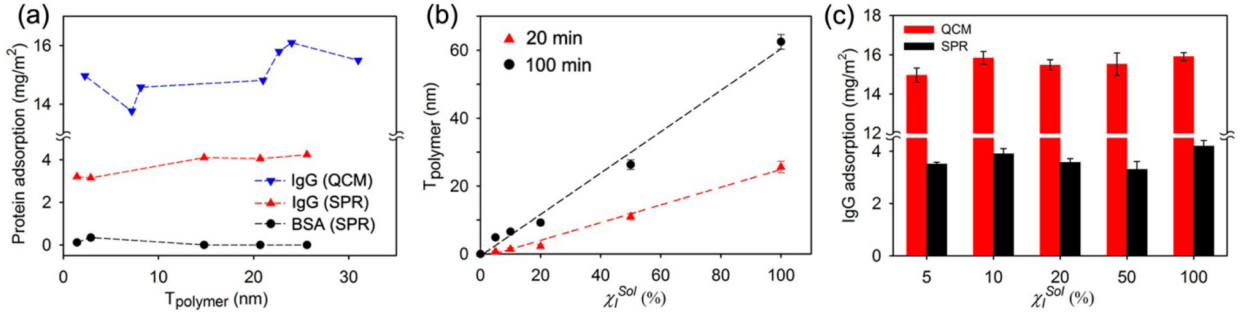

Figure 2. Protein adsorption on poly(AMA-*co*-HEMA)−peptide layers as a function of their thicknesses ($T_{polymer}$) and the graft density (as accounted for by χ_I^{Sol}) (Figure 2c). (a) QCM and SPR results of protein adsorption as a function of thickness; (b) Development of the thickness of poly(AMA-*co*-HEMA) ($T_{polymer}$) as a function of χ_I^{Sol} (χ_I^{Sol} from 5 to 100%) for a polymerization time of 20 min (red triangles) or 100 min (black circles). (c) IgG adsorption on poly(AMA-*co*-HEMA)−peptide systems as a function of χ_I^{Sol} for the polymers grown during 20 min of polymerization (SPR) and 100 min (QCM).

but different $T_{polymer}$ were prepared by changing the polymerization time.[33] After peptide immobilization, the distribution of peptide in the matrix was evaluated. It was found that peptide immobilization resulted in a linear increase of the ellipsometric thickness of the polymer layer ($\Delta T_{polymer}$, Figure 1a, circle symbols), which can be described by the following relationship (R^2 > 98%):

$$\Delta T_{polymer} = 0.29 T_{polymer} \quad (1)$$

The amount of peptide immobilized to the surface was further examined by QCM knowing that the increase of effective mass in air can be calculated from the decrease of frequency using the Sauerbrey equation:

$$\Delta m = -\frac{C\Delta f_n}{n} \quad (2)$$

where Δm is the increase of QCM mass, Δf_n is the frequency shift at a given overtone number n (n = 3 was used in this study), and C is a constant, 17.7 ng/cm^2 Hz^{-1} for the AT-cut 5 MHz quartz crystals used in these experiments. It was found that the mass of immobilized peptide on the polymer also increased linearly with increasing $T_{polymer}$ (Figure 1a, triangle symbols) as described by the following relationship (R^2 = 97%):

$$\Delta m_{peptide} = 0.45 T_{polymer} \quad (3)$$

where $\Delta m_{peptide}$ is the area mass of immobilized peptide (mg/m^2).

These linear relationships (eqs 1 and 3) suggest that the short peptides diffused effectively into the polymer layer, regardless of its thickness. This hypothesis is further confirmed by XPS analyses at different takeoff angles of the poly(AMA-*co*-HEMA)−peptide surface with $T_{polymer}$ = 23.9 nm and χ_I^{Sol} = 100%. We observed that the percentage of nitrogen increased from 1.7% for the poly(AMA-*co*-HEMA) surface[33] to 8.2% after peptide immobilization, and no apparent changes in the % density of C, N, and O were observed for the peptide surface probed at different takeoff angles (Figure 1b); these facts suggest that the binding peptides in the polymer matrix were distributed uniformly at least within the topmost 10 nm layer thickness (equivalent to the XPS sampling depth).

The adsorption of BSA and IgG on poly(AMA-*co*-HEMA)−peptide surfaces with different $T_{polymer}$ was measured by SPR (Figure 2a). In the range of polymer thickness from ∼1.5 to ∼25.6 nm, all the surfaces exhibit excellent nonspecific BSA resistance (less than 0.2 mg/m^2) and can reach almost zero adsorption for surfaces with $T_{polymer}$ > 15 nm. While as $T_{polymer}$ increases, IgG binding increases gradually and almost reaches saturation when $T_{polymer}$ is above 15 nm. The maximum IgG adsorption is ∼4.2 mg/m^2, which is very similar to the level of IgG adsorption onto Ac-HWRGWV peptide modified chromatographic resins (4.5 mg/m^2).[12] The specific binding of IgG to the surfaces with different $T_{polymer}$ was also

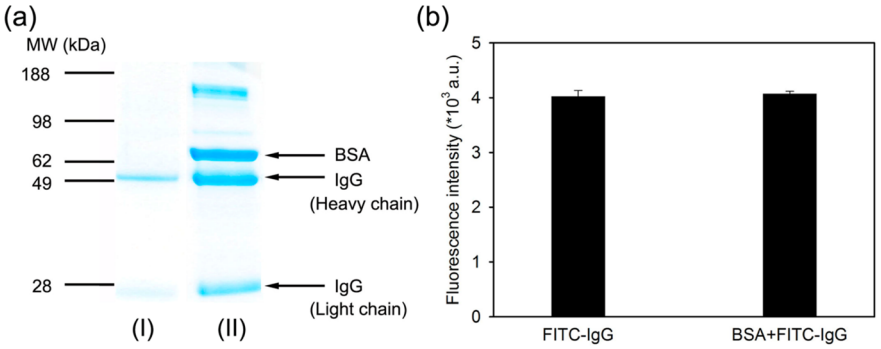

Figure 3. Specific adsorption of IgG to peptide systems in the presence of interfering BSA. (a) SDS-PAGE of elution fractions of proteins from the poly(AMA-*co*-HEMA)−peptide surface after incubation in mixed solution containing 1 mg/mL IgG and 1 mg/mL BSA (I). Signals corresponding to proteins from mixed solution containing 1 mg/mL IgG and 1 mg/mL BSA are shown in (II). Labels: MW, molecular weight marker. (b) Fluorescence intensity after adsorption of FITC−IgG onto the peptide surface from 0.2 mg/mL FITC−IgG solution with and without 0.2 mg/mL BSA.

investigated by QCM. The corresponding adsorbed mass calculated by the "solidified liquid model" was recorded (Figure 2a, inverted triangle symbols). It is observed that a gradual increase in IgG binding occurs with increasing $T_{polymer}$. It is noted that the difference of adsorption measured by SPR and QCM is due to the coupled water with IgG molecules.[34]

The limited influence of polymer thickness on IgG binding is quite different from suggestions derived from data presented in Figure 1, which indicated that the amount of peptides immobilized on the polymer increased linearly with an increased $T_{polymer}$. Therefore, it is suggested that not all of the immobilized peptides are available for interaction with IgG. In contrast to the small peptide, which can penetrate and diffuse easily into the polymer layer, IgG is a large molecule (14.5 nm × 8.5 nm × 4 nm)[45] and thus the binding of IgG is influenced by steric hindrance.[46] On the basis of these results, we suggest that IgG−peptide binding occurs mainly on the topmost layer of the supporting polymer matrix. In addition, the IgG adsorbed on the topmost region of the polymer layer is likely to block further access of incoming IgG molecules to the interior of the polymer matrix. This limited binding due to restricted penetration of biomolecules throughout the highly packed brush layer is also reported for other systems.[40,47] A reasonable question is therefore if such a limitation can be offset by reducing the graft density, an issue that is discussed next.

We used binary mixed SAMs to adjust the surface density of initiator and thus the density of grafted polymer chains. The solution ratio of initiator (χ_I^{Sol}) was used to describe the final surface density of initiator (χ_I^{Surf}), since it can be assumed that these two variables are correlated (even if not linearly, depending on the case).[48] As such, a series of surfaces with varying χ_I^{Sol}, from 5 to 100%, were prepared within different polymerization times (20 or 100 min). An approximately linear increase in thickness of grafted polymer ($T_{polymer}$) with increasing χ_I^{Sol} was observed regardless of polymerization time (Figure 2b), in agreement with previous reports.[32] We note that SPR is not suitable for measurements of thick polymer layers due to the high optical density (in this experiment, the polymer thickness is above 35 nm). Therefore, the IgG adsorption on the poly(AMA-*co*-HEMA)−peptide surfaces was determined by SPR only for thin support layers (polymerization time of 20 min), and QCM was used for thicker samples (with a polymerization time of 100 min). It is found that no significant changes in IgG adsorption occur when comparing the surfaces prepared under the same polymerization time, regardless of χ_I^{Sol}. This suggests a limited influence of the graft density of the polymer matrix on the binding capability of IgG.

Thus, in the present system, decreasing the graft density does not enhance IgG binding. It should be noticed that, under lower χ_I^{Sol}, the grafted polymer layers are thin even for polymerization times of 100 min; this is mainly due to the limited polymerization thickness for AMA with the ATRP method,[49] which leads to reduced AMA segments that provide the NH_2 groups for peptide immobilization. We also prepared self-assembled monolayers (SAMs) consisting of SH-$(CH_2)_{11}(CH_2CH_2O)_6NH_2$ for peptide immobilization and investigated the IgG binding on this system by SPR.[50] The results indicated that IgG adsorption on the SAM−peptide system was slightly lower than that on the poly(AMA-*co*-HEMA)−peptide surface; however, the BSA adsorption on the SAM, 2-D system is much higher than that on the 3-D polymer matrix (0.5 mg/m^2 vs almost zero). We attribute the better resistance to nonspecific interactions to the 3-D structure of the polymer matrix and the presence of HEMA segments, which promote steric repulsion and form a hydration layer that limits or prevents nonspecific protein adsorption. Since a biosensor matrix generally requires effective immobilization of bioactive molecules (at large densities for enhanced detection) and should also prevent nonspecific interaction (for reduced interference), here we adopted 3-D polymer layers as a sensing platform.

Specific Adsorption of IgG in the Presence of Interfering BSA. In order to test the biospecific nature of the peptide−IgG binding, we conducted adsorption experiments by incubating the poly(AMA-*co*-HEMA)−peptide surface in IgG/BSA mixed solution and evaluated the extent of protein adsorption via SDS-PAGE and fluorescence microscopy.

After 2 h of exposure to IgG/BSA mixed solution, the adsorbed proteins on the surface were eluted by 2% SDS and ran on reduced SDS-PAGE gels, so as to separate the proteins according to molecular weight (band I, Figure 3a). The proteins from the mixed solution were used as a control (band II, Figure 3a). Strong signals were detected at about 66, 50, and 25 kDa on band II, which correspond to the BSA, and the heavy and the light chain of IgG, respectively. On band I, only signals at 50 and 25 kDa were observed, indicating that the poly(AMA-*co*-HEMA)−peptide surface can selectively bind

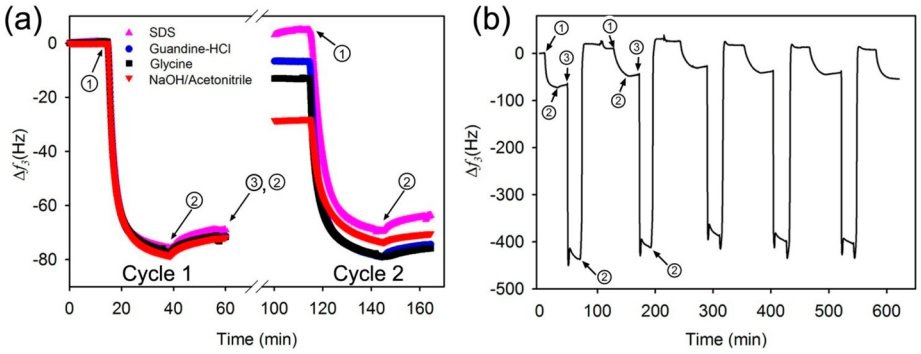

Figure 4. Regeneration of peptide-modified poly(AMA-*co*-HEMA) layers with (a) four different elution buffers, (b) 2% SDS solution. The adsorbed IgG can be easily eluted after SDS injection, resulting in the regeneration of the active polymer layers. ①–③ stand for the injection of ① IgG, ② PBSS in (a) or PBS in (b) and ③ elution buffer.

IgG and resist BSA adsorption. Furthermore, we used fluorescence microscopy to test the FITC–IgG adsorption on the peptide surface ($T_{polymer}$ = 32 nm and χ_I^{Sol} = 100%) with and without BSA. The corresponding fluorescence intensities indicate no significant differences (Figure 3b) and suggest the absence of interference effects from the presence of BSA. Taking it all together, it is concluded that the poly(AMA-*co*-HEMA)–peptide surface can selectively bind the target IgG molecule from the mixed protein solution while maintaining nonspecific resistance.

Reproducibility and Regenerability of Poly(AMA-*co*-HEMA)–Peptide Systems. Reproducibility and regenerability are important criteria for the development of biosensors for practical deployment, which could facilitate rapid testing of multiple samples at a reduced cost.[51,52] Compared with other common IgG ligands such as protein A/G, short peptides have a relatively lower binding affinity to IgG,[33] making it easier for the regeneration of the sensor. The goal in regeneration steps of the peptide-based sensor is to offset the affinity between IgG and peptide and remove the adsorbed IgG using proper elution reagents without destroying the peptide activity. Acid, base, or salt solutions of high ionic strength have been used as elution buffer to desorb IgG from surfaces.[14,51,52] In addition, it has been noted that a combination of different chemicals is favorable for dissociating specifically bound biomolecules. In this study, several different eluting reagents including 6 M guanidine–HCl, 2% SDS, 0.2 M glycine–HCl buffer, and mixed solution of 0.1 M NaOH with 10% acetonitrile were chosen to investigate their elution ability of adsorbed IgG.

Figure 4a shows the adsorption and elution of IgG from peptide systems as measured by QCM. The injection of IgG induces a repeatable decrease of frequency, by −71 ± 1 Hz in cycle 1. This level of binding indicates that the short peptide sensors have a reproducible performance. After injection of the different elution buffers and re-equilibration with PBSS (also used to produce a new baseline for cycle 2 of IgG injection), the frequency increases to 5, −7, −13, and −28 Hz for SDS, guanidine–HCl, glycine–HCl, and NaOH with 10% acetonitrile, respectively, suggesting that all of these buffers can at least partly elute IgG from the surface. Among them, SDS is the most effective elution system to regenerate the surface back to the original condition. We found that the treatment of SDS leads to a final frequency a little higher than the initial baseline (0 Hz), which may be because some peptides physically bound to the poly(AMA-*co*-HEMA) were solubilized by SDS.

The regenerability of the peptide-based system after the treatment of elution buffer was evaluated by successive injections of IgG over the peptide surface. The binding of IgG in cycle 2 induced a decrease in QCM frequency to −69, −68, −62, and −42 Hz for SDS, guanidine–HCl, glycine–HCl, and NaOH with 10% acetonitrile, respectively. If the % degree of regenerability is quantified by the ratio of frequency decrease in cycle 2 to that in cycle 1, the following figures are obtained: 97, 96, 87, and 59% after using SDS, guanidine–HCl, glycine–HCl, and NaOH with 10% acetonitrile as elution buffer of the respective surfaces. Compared with other elution buffers, SDS exhibits the highest regenerability (97%); therefore, it can be used efficiently for peptide regeneration.

We found that the injection of 2% SDS to PBSS directly forms a precipitate and thus easily blocks the QCM tubing. Therefore, instead of PBSS, PBS was used as a running buffer to test the regenerability of the system by SDS and thus to allow use in multiple cycles (Figure 4b). It is found that, after running up to 6 cycles, only 6% activity loss was detected for the same peptide sensor, suggesting that the system can be used for a series of measurements after regeneration with SDS.

Storage Stability of Poly(AMA-*co*-HEMA)–Peptide Systems. Storage stability is another important requirement in biosensors.[53] The decrease of sensitivity due to the denaturation of protein-based ligands (such as protein A) is a problem in typical IgG sensors used in long-term applications.[54] In contrast, the short peptide ligand in the proposed system is a small molecule with a stable structure and thus may maintain activity for longer times. For the storage stability studies, a set of poly(AMA-*co*-HEMA)–short peptide systems were prepared under identical conditions and stored in air or PBS at 4 °C for different time periods. The response to IgG was investigated by QCM, as shown in Figure 5. After up to 20 days storage under both conditions (in air or in PBS), the sensor yields a similar response to that when tested the first day, while the QCM signals of sensors stored in air were slightly higher than those stored in PBS. Overall, the results indicate that the poly(AMA-*co*-HEMA)–peptide system has good stability and could be used for a long time under proper storage conditions.

CONCLUSIONS

The interfacial activities of a random copolymer of poly(AMA-*co*-HEMA) carrying immobilized Ac-HWRGWVA peptides was investigated as a system to selectively bind IgG. IgG molecules adsorbed onto the topmost layer of the polymer layer. The graft density and thickness of the supporting polymer slightly

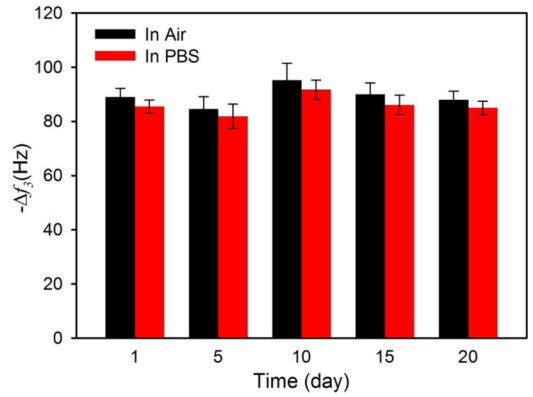

Figure 5. IgG adsorption on poly(AMA-*co*-HEMA)−peptide sensors stored in air and in PBS at 4 °C for different time periods.

affected the binding capability of IgG. The proposed peptide-based system exhibits good specificity to target IgG molecule even in the presence of interfering proteins. Moreover, the short peptide system was used repeatedly with very small losses of activity (6% loss of activity after repeated use for six times when using 2% SDS as elution buffer). The sensing system is stable and maintains its bioactivity up to 20 days when stored in air at 4 °C. These features indicate that the poly(AMA-*co*-HEMA)−peptide system holds potential for low cost, robust, reliable, and reusable devices for detection of IgG.

AUTHOR INFORMATION

Corresponding Author

*E-mail: ojrojas@ncsu.edu (O.J.R.); yzhang58@ncsu.edu (Y.Z.). Phone: +1-919-513 7494 (O.J.R.). Fax: +1-919-515 6302 (O.J.R.).

Notes

The authors declare no competing financial interest.

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