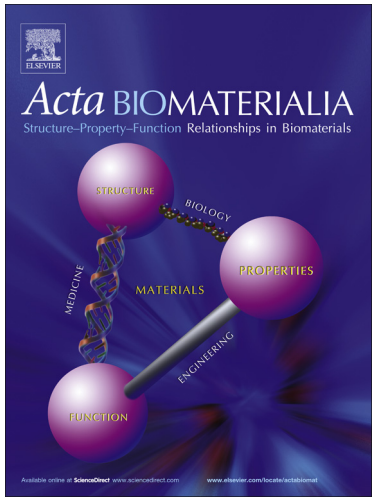

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Ultra-Low Fouling and High Antibody Loading Zwitterionic Hydrogel Coatings for Sensing and Detection in Complex Media

Ying-Nien Chou,[†,‡,§] Fang Sun,[†,§] Hsiang-Chieh Hung,[†] Priyesh Jain,[†] Andrew Sinclair,[†] Peng Zhang,[†] Tao Bai, [†] Yung Chang,[⊥] Ten-Chin Wen*,[‡] Qiuming Yu,[†] Shaoyi Jiang*[†]

[†]Department of Chemical Engineering, University of Washington, Seattle, Washington 98195-1750, USA

[‡]Department of Chemical Engineering, National Cheng Kung University, Tainan 70101, Taiwan

[⊥]R&D Center for Membrane Technology and Department of Chemical Engineering, Chung Yuan Christian University, Chung-Li, Taoyuan 320, Taiwan

§These authors contributed equally to this work

* Corresponding author. Department of Chemical Engineering, University of Washington, Seattle, Washington 98195, USA. Phone: 1-206-616-6509, Fax: 1-206-543-3778. E-mail: sjiang@uw.edu (S. Jiang).

** Corresponding author. Department of Chemical Engineering, National Cheng Kung University, Tainan 70101, Taiwan. Phone: 886-3-265-4122, Fax: 886-3-265-4199. Email: cwen@mail.ncku.edu.tw (T.C. Wen)

Abstract

For surface-based diagnostic devices to achieve reliable biomarker detection in complex media such as blood, preventing nonspecific protein adsorption and incorporating high loading of biorecognition elements are paramount. In this work, a novel method to produce nonfouling zwitterionic hydrogel coatings was developed to achieve these goals. Poly(carboxybetaine acrylamide) (pCBAA) hydrogel thin films (CBHTFs) prepared with a carboxybetaine diacrylamide crosslinker (CBAAX) were coated on gold and silicon dioxide surfaces via a simple spin coating process. The thickness of CBHTFs could be precisely controlled between 15 and 150 nm by varying the crosslinker concentration, and the films demonstrated excellent long-term stability. Protein adsorption from undiluted human blood serum onto the CBHTFs was measured with surface plasmon resonance (SPR). Hydrogel thin films greater than 20 nm exhibited ultra-low fouling (<5 ng/cm^2). In addition, the CBHTFs were capable of high antibody functionalization for specific biomarker detection without compromising their nonfouling performance. This strategy provides a facile method to modify SPR biosensor chips with an advanced nonfouling material, and can be potentially expanded to a variety of implantable medical devices and diagnostic biosensors.

KEYWORDS: hydrogel coatings; zwitterionic polymers; carboxybetaine; sensing and diagnostics; complex media.

1. Introduction

Avoiding nonspecific protein adsorption is critical in numerous fields. Biosensors, implanted medical devices, drug delivery systems and ship hulls all benefit tremendously from nonfouling surface chemistries [1-4]. Many materials have been reported to reduce protein fouling, but key challenges remain. Although poly(ethylene glycol) (PEG) and its derivatives are the most widely used nonfouling materials, they are susceptible to oxidative damage over long-term use, difficult to directly functionalize with biomolecules for biosensing applications, and have limited nonfouling capabilities in complex, real-world media such as undiluted blood plasma and serum [5, 6]. Zwitterionic materials such as phosphorylcholine (PC), sulfobetaine (SB) and carboxybetaine (CB) are attractive alternatives and have seen increasing investigation [7-11]. Poly(carboxybetaine) materials, including poly(carboxybetaine methacrylate) (pCBMA) and poly(carboxybetaine acrylamide) (pCBAA), are particularly interesting. They exhibit ultra-low fouling properties (i.e., <5 ng/cm^2 of nonspecific protein binding) in 100% blood plasma and serum [12, 13]. In addition, abundant carboxylic acid groups make antibody functionalization to pCB straightforward via amino coupling chemistries [14]. These materials have proven useful for cancer diagnosis in whole blood [14, 15].

Typical methods of coating pCB on surfaces can be classified into two categories: “graft-from” and “graft-to”. In the “graft-from” approach, initiators are first immobilized on a substrate and a polymerization method such as atom transfer radical polymerization (ATRP) is used to grow polymer chains from the surface-bound initiators [8]. The “graft-to” method involves direct attachment of zwitterionic polymers—containing adhesive groups such as DOPA or thiols—onto the target surface [16, 17]. While the “graft-from” technique is an excellent strategy to achieve a high packing density and controlled film thickness, “graft-to” is a more convenient coating process. However, the “graft-from” ATRP often requires oxygen-free conditions while the “graft-to” surface-adhesive polymers require extensive coating optimization. In addition, these quasi-2D coatings with high surface packing densities have limited space for subsequent protein immobilization [18]. Thus, new facile strategies to achieve nonfouling coatings with high protein loadings are highly desirable.

Hydrogel coatings are simple and convenient to attain, and several studies have examined their usage for protein resistance and biosensor functionalization [19-25]. The aforementioned PEG is regularly used to generate hydrogels for biomedical applications via photo-initiated

crosslinking. Although PEG hydrogels resist protein adsorption, they fall short of achieving ultra-low protein adsorption from undiluted blood plasma or serum [26-28]. Polysaccharide-based materials such as dextran have also been reported to form hydrogel coatings on biosensors through a number of different methods, but these coatings could not stand with undiluted blood plasma or serum [29, 30]. A zwitterionic hydrogel film containing phosphorylcholine (PC) groups has been investigated for its swelling properties, but its tolerance to complex media has not been reported [31-33]. Fully zwitterionic hydrogels assembled from a CB monomer and a CB-based crosslinker exhibit many unique qualities—they have been demonstrated to resist nonspecific protein adsorption in undiluted whole blood [34], to prevent the foreign-body reaction to implanted materials [35], and to restrain the differentiation of mesenchymal stem cells [36]. In addition, the three-dimensional (3D) structure of the gel matrix provides many sites amenable to ligand immobilization for the specific detection of biomolecules. These virtues suggest that a pCB-based hydrogel coating may be up to the challenge of resisting protein fouling from complex, real-world media and providing abundant functional groups for the subsequent immobilization of molecular bio-recognition elements while maintaining coating stability and simplicity. To the best of our knowledge, a simple hydrogel coating resisting nonspecific protein adsorption from undiluted blood serum has not been reported.

In this work, we develop a nonfouling, wholly zwitterionic coating based on a pCBAA hydrogel thin film (CBHTF). As shown in **Scheme 1**, a new zwitterionic CB-based diacrylamide crosslinker (CBAAX) was synthesized for this work. The hydrogel thin films were formed by spin-coating a stock solution containing monomer, crosslinker, and photoinitiator onto a hydrophilic, self-assembled monolayer (SAM)-modified gold surface, followed by ultraviolet (UV) light irradiation. The thicknesses of CBHTFs could be controlled by varying the crosslinker concentration, and the coatings displayed great stability. Optimized formulations of CBHTFs demonstrate ultra-low fouling (<5 ng/cm^2) from 100% human blood serum as measured by surface plasmon resonance (SPR), which has not been previously reported for a hydrogel coating. The CBHTF coating also performed well on a SiO_2-modified SPR sensor, indicating its capability for general use. CBHTF-coated SPR sensor chips could further be functionalized with antibodies for specific biomarker detection via the simple 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide/N-hydroxysuccinimide (EDC/NHS) coupling chemistry, which benefits from abundant carboxylic acid groups in the 3D CBHTF matrix. Antibody

immobilization and biomarker detection levels were also optimized by controlling the crosslinking density.

2. Experimental Section

2.1 Materials

CBAA monomers were synthesized following a previously published method [37]. Acryloyl chloride, acetonitrile, tert-Butyl bromoacetate, dichloromethane (DCM), 2-Hydroxy-2-methylpropiophenone (HMPP), 11-Mercapto-1-undecanol ($C_{11}OH$), 1-Decanethiol (C_{11}), 12-Mercaptododecanoic acid ($C_{11}COOH$) and phosphate-buffered saline (PBS) were purchased from Sigma Aldrich (St. Louis, MO). 2,2'-Diamino-N-methyldiethylamine and N,N-Diisopropylethylamine (DIPEA), and trifluoroacetic acid (TFA) were purchased form TCI (Portland, OR). Ethanol was purchased from Decon Labs (King of Prussia, PA). Sodium acetate anhydrous was purchased from Fluka (subsidiary of Sigma Aldrich, St. Louis, MO). EDC and NHS were purchased from Acros Organics (Geel, Belgium). Pooled human serum was purchased from Biochemed Services (Winchester, VA). Human thyroid stimulating hormone (TSH) antibody and antigen were from Thermo Scientific (Waltham, MA). The buffers including 150 mM phosphate buffered saline (PBS) at pH 7.4, 10 mM sodium acetate buffer (SA) at pH 5, 10 mM boric acid (BA) with 300 mM NaCl at pH 9, 10 mM glycine (GLY) at pH 4 and 10 mM HEPES at pH 7.5 were prepared for biomolecular conjugation and bioassay. The pH values were adjusted with HCl or NaOH, and buffers were degassed prior to use. All water used was purified to 18.2 MΩ with a Millipore water purification system (Billerica, MA).

2.2 Synthesis of carboxybetaine diacrylamide crosslinker

A synthesis schematic of the CBAAX is shown in **Scheme 2**. A solution of acryloyl chloride (5.3 mL, 65.1 mmol) in 20 mL DCM was added dropwise to a solution of 2,2'-Diamino-N-methyldiethylamine (4 mL, 31.0 mmol) and DIPEA (11,9 mL, 68.3 mmol) in 50 mL DCM at 0℃ over a 30 min period. The reaction mixture was allowed to warm to room temperature and stirred for two hours. The reaction was then washed with (2 x 25 mL) water. The aqueous layer was re-extracted with DCM (3 x 75 mL). The combined organic layers were dried using sodium sulfate, filtered, and concentrated in vacuo to leave a residue which was further purified by column chromatography to give compound **2** (5.2 gm) in 75.3% yield. ^{1}H NMR (300 MHz, D_2O) δ 6.02 – 5.97 (m, 4H), 5.56 – 5.51 (m, 2H), 3.14 (t, J = 6.7 Hz, 4H), 2.37 (t, J = 6.7 Hz, 4H), 2.07 (s, 3H).

Compound **2** (3.2 gm, 10.5 mmol) was dissolved in 20 mL acetonitrile and tert-butylbromoacetate (4.2 mL, 31.6 mmol) was added to it. The reaction contents were stirred for 18 hours at 65^{o}C until TLC showed complete consumption of the starting material. The reaction mixture was concentrated to dryness in vacuo and further purified by column chromatography to give compound **3** (4.3 gm) in 72.6% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.31 – 6.08 (m, 4H), 5.67 (dd, $J_1 = J_2 = 2.4$ Hz, 2H), 4.48 (s, 2H), 3.75 – 3.54 (m, 8H), 3.29 (s, 3H), 1.45 (s, 9H).

Compound **3** (2.0 gm, 4.7 mmol) was dissolved in 15 mL DCM and 15 mL TFA was added. The reaction contents were stirred overnight at room temperature. After complete hydrolysis, the reaction mixture was concentrated in vacuo and co-evaporated with DCM (3 x 15 mL). The resulting residual mixture was further dissolved in 15 mL methanol and 4 mg IRN-78 resin was added to it for complete neutralization. The residue was dissolved in water and lyophilized to give compound **4** in 84.1% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.31 – 6.05 (m, 4H), 5.63 (dd, $J_1 = J_2 = 2.4$ Hz, 2H), 3.70 (s, 2H), 3.69 – 3.50 (m, 8H), 3.21 (s, 3H).

2.3 Preparation of CBAA hydrogel solution and coating on SPR sensor chips

Monomer solutions were prepared in DI water, at a concentration of 9% by weight. The crosslinker CBAAX was added to these solutions in quantities ranging from 0.5 to 20% (molar percent of monomer). A 2.5% (molar percent of monomer) solution of photoinitiator HMPP was then added, and complete solutions were sonicated before spin-coating. The SPR sensor chips were produced by coating a glass slide with a 2 nm titanium film followed by a 50 nm gold film using an electron beam evaporator. The gold SPR chips were cleaned and modified with different SAMs by soaking the chips in ethanol solutions containing specified thiols (1 mM) overnight. The chips were then rinsed with DI water and ethanol and dried in a stream of filtered air. The prepared monomer solutions were spin-coated on the cleaned chips at 3000 rpm for 50 s. The spin-coated film was dried with filtered air and the chips were exposed to UV light (6 W; 302 nm with 280 nm cutoff filter) for 120 min to commence polymerization. After UV crosslinking, the hydrogel-coated substrates were immersed in PBS solution for 3 days to allow the hydrogel films to fully swell and remove unreacted reagents. The same CBHTF coating process was conducted on the SiO_2-modified SPR chips. Before spin-coating, the SiO_2 surface was washed by immersion in piranha solution for 15 mins followed by ammonium hydroxide solution for 15 mins to generate hydroxyl groups. The chips were then washed with DI water and dried with filtered air. The CBHTF was directly coated on the SiO_2 surface without SAM modification.

2.4 Film thickness and film stability analysis

The thickness of CBHTFs were determined using an ellipsometer, (Model alpha-SE, J.A. Woollam, Lincoln, NE) using a 380–900 nm wavelength range at an incidence angle of 70°. The results were fitted to a Cauchy model. Four locations on each sample were analyzed to ensure uniformity of the hydrogel films. The stability of CBHTFs was studied by monitoring changes in thickness after immersion in PBS solution and shaking for 12 hrs, 24 hrs, 3 days and 30 days. Before each thickness measurement, CBHTF-modified chips were rinsed with DI water and dried with filtered air. All the thicknesses were measured under dry condition unless otherwise specified.

2.5 Nonspecific protein adsorption, antibody immobilization, and antigen detection

Nonspecific protein adsorption, antibody immobilization, and antigen detection were monitored using a custom-built four-channel SPR sensor as described previously [38]. To measure protein adsorption, undiluted human serum was injected (10 min, 40 μL/min) and the wavelength shift between PBS baselines was converted to a surface coverage. Anti-TSH was immobilized by first injecting 10 mM SA (pH 5), followed by EDC/NHS (0.2 M/0.05 M in water) for 7 min at 30 μL/min. Then, anti-TSH (50 μg/mL in 10 mM HEPES pH 7.5) was injected (20 min, 20 μL/min) followed by deactivation with 10 mM BA (pH 9) for 10 min, then 10 mM GLY (pH 4) and SA for 10 min, all at 30 μL/min. Immobilization was calculated as the difference between SA baselines before Anti-TSH injection and after deactivation. TSH antigen binding was then monitored by first injecting PBS and then antigen (1 μg/mL in PBS at 40 μL/min) following by PBS.

2.6 Statistical methods

Statistical analysis was performed using Excel. Mean values with standard deviation are reported and all experiments were performed in triplicate. The error bars correspond to the standard deviation

3. Results and Discussion

3.1 Synthesis of CBAAX and selection of SAM modification on gold chips

Crosslinker selection plays an important role in the nonfouling performance of zwitterionic hydrogels [39]. Zwitterionic materials repel nonspecific protein adsorption through strong binding to water via electrostatically induced hydration. However, traditional crosslinkers such as widely-used N,N'-methylenebis(acrylamide) (MBAA) are only moderately water soluble

(not more than 10%). They can degrade the nonfouling performance and mechanical strength of the hydrogel system. To overcome this problem, we have synthesized a CBAAX (**Scheme 2**) for improved solubility, homogeneity, mechanical properties and compatibility with CBAA monomer to form CBAA-based zwitterionic hydrogel coatings.

CBHTF coatings were deposited by spin-coating aqueous hydrogel solutions onto gold chips, followed by photoinitiated polymerization. Notably, we found CBHTF uniformity to be related to the hydrophilicity of a chip surface. Grains and particles were observed in the CBHTF after initial coating on a cleaned bare gold SPR chip. Therefore, further chips were modified with one of several SAMs, including C_{11}COOH, C_{11}OH, and $C_{11,}$ to alter surface hydrophilicity and form a smoother film. CBHTFs could be successfully coated on C_{11}COOH and C_{11}OH SAM-modified gold chips with relatively good uniformity, while they could not be formed on the C_{11} SAM-modified chip used as a negative control. **Table 1** shows the water surface contact angles of different SAM-modified gold chips for their hydrophilicities. The hydrophobic C_{11} SAM-modified substrate presented a 78.3º contact angle, and strongly repelled the attachment of the aqueous hydrogel solution as expected. The carboxyl tail groups present in the C_{11}COOH SAM resulted in a very low contact angle (~5º), even compared with that of the C_{11}OH SAM (~16º). On this C_{11}COOH SAM, the CBHTF demonstrated the best uniformity, greatest film thickness and best nonfouling performance. We hypothesize that the fouling resistance of CBHTFs is highly dependent on their surface uniformity, and thus hydrophilic pre-modification of gold chips is necessary for optimal performance. Due to the excellent uniformity of films grown on C_{11}COOH SAM-modified chips, we selected this SAM for subsequent experiments to optimize nonfouling and test antibody immobilization.

3.2 Control of CBHTF thickness and stability test

The thickness of CBHTFs could be precisely controlled by varying the CBAAX concentration in the stock hydrogel solution. The CBAA monomer concentration was fixed at 9 wt%, and the CBAAX crosslinker was added in ratios from 0 to 20 mol% of the CBAA monomer. As shown in **Figure 1a**, CBHTF thickness increases with an increased crosslinker ratio because of enhanced accumulation of the 3D polymer network. The film thickness in the absence of crosslinker was only 12 nm. In the presence of CBAAX, well-controlled film thicknesses ranging from ~15 to ~40 nm could be attained with CBAAX ratios ranging from 0.5% to 6%, and ~90 to ~150 nm thicker films were achieved with higher CBAAX ratios between 10 and

20%. Film thicknesses showed higher variations when 20% CBAAX was used, as the reaction was more difficult to control under this high crosslinker ratio. These two film thickness regions are both desirable for different coating applications.

Figure 1b shows the stability of CBHTFs immersed in PBS for different periods of time. The thickness of CBHTFs containing 20% CBAAX only decreased slightly after immersion for 30 days, from 145 nm to 142 nm. Meanwhile, CBHTFs formulated with 3% and 6% CBAAX showed even better stability, with no obvious change in film thickness. However, the thickness of the CBAA polymer brush film grafted without CBAAX crosslinker decreased from 12 nm to 1 nm over one month. These results emphasize how important crosslinker selection is to achieve stable films. With CBAAX incorporation, robust and well-controlled CBHTF coatings were achieved using this simple coating method.

3.3 Protein resistance test in human blood serum

To challenge CBHTFs with complex media, we used an SPR sensor to measure protein adsorption from undiluted human blood serum onto CBHTFs of varying thicknesses coated on C_{11}COOH-modified gold chips. **Figure 2** shows this protein adsorption from 100% serum as a function of CBHTF thickness. High fouling (~40-70 ng/cm^2) was seen at a CBHTF thickness of less than 20 nm. However, when the CBHTF thickness was over 25 nm, protein adsorption dropped to an ultra-low fouling level (<10 ng/cm^2). The lowest fouling observed was less than 5 ng/cm^2 at a film thickness of 29.7 nm. The maximum film thickness tested was around 45 nm since the SPR detection sensitivity drops exponentially when away from the surface. The higher level of protein adsorption seen onto 45-nm-thick CBHTFs was attributed to a reduced film uniformity compared with those of lower thicknesses. For pCB brush coatings, a high packing density of zwitterionic moieties is the key to achieving strong hydration and effective nonfouling performance. For CBHTFs, the crosslinking density plays an important role in surface nonfouling properties. Utilizing higher concentrations of CBAAX (> 2%) allowed us to increase both the film thickness and the degree of crosslinking in a controlled fashion. To test CBHTF performance on a different hydrophilic surface other than COOH SAM, we modified chips with SiO_2 before hydrogel coating and serum challenge. **Figure 3** shows a typical SPR sensorgram of protein adsorption from undiluted blood serum on these SiO_2-modifed chips coated with a 27.2 nm-thick CBHTF. Less than 5 ng/cm^2 of protein adsorption was seen for these chips as well, validating the broad applicability of this coating method. For both types of modified chips,

optimizing the crosslinking density is a key to achieving ultra-low fouling.

3.4 Surface functionalization and detection

It has been demonstrated previously that zwitterionic pCB-based coatings possess abundant functionalizable groups that enable antibody immobilization via NHS/EDC amino-coupling chemistries, and that these coated surfaces can maintain an ultra-low fouling background before and after antibody functionalization [27]. However, these pCB coatings are typically grafted via ATRP, and are quasi-two-dimensional (2D) films intrinsically limited to a maximum ligand immobilization capacity of a monomer (~250 ng/cm^2). In contrast, the CBHTFs developed in this work comprise a 3D matrix with CB moieties on each monomer *and* crosslinker unit, enhancing the functionalization capacity well beyond that of 2D films.

To study the antibody immobilization capacity of CBHTFs, we functionalized the films with antibodies against human thyroid stimulating hormone (anti-TSH). Antigen binding and nonspecific protein from undiluted human serum were investigated using SPR. **Figure 4** shows a typical SPR sensorgram of the overall process, i.e., surface activation, antibody immobilization, surface deactivation and antigen detection as conducted on a CBHTF with 4% CBAAX content as described in the experimental section. Using SPR, we systematically investigated the influence of crosslinking density (i.e., the CBAAX ratio) on antibody loading capacity and antigen detection. As shown in **Figure 5,** the optimal antibody loading was around 693 ng/cm^2 at a CBHTF thickness of ~28 nm containing 3% CBAAX, a threefold higher loading capacity than that of 2D pCB films previously reported [18]. We also measured the wet thickness of the optimal CBHTF; it had swelled from ~28 nm to ~63 nm, indicating its 3D structure. Notably, the loading capacity decreased to 411 ng/cm^2 in a thicker ~40 nm film. The higher crosslinker content rendered the gel network denser, inhibiting the diffusion of antibodies to binding sites. The antigen (i.e., TSH) binding capacity in the optimized ~28 nm film was 68.5 ng/cm^2, corresponding to a calculated bioactivity ratio of 0.71. We also tested the nonspecific fouling resistance of the optimized CBHTF after antibody immobilization, and found it to remain under 5 ng/cm^2 from undiluted serum.

In this work, the most important factor to achieve ultra-low fouling and high antibody loading was the crosslinker ratio. Once the crosslinker ratio was above 2%, the CBHTFs demonstrated good stability and showed relatively low protein adsorption due to the higher crosslinking density. However, the optimized crosslinker ratio for maximum antibody loading

was in the middle of the range, as higher crosslinker content could make the CBHTF too dense and inhibit antibodies from diffusing in. These results indicate that control over the architecture of zwitterionic hydrogel thin films is the key to their unique capacity for ultra-low nonspecific protein adsorption with high ligand loading.

4. Conclusions

In summary, we have developed a facile approach to realize ultra-low fouling and high ligand loading with a highly-crosslinked, purely zwitterionic, carboxybetaine thin film hydrogel (CBHTF) coating platform. The CBHTF on a hydrophilic surface demonstrated long-term stability. By varying the crosslinker content in the spin-coated hydrogel solution, the thickness of CBHTFs could be precisely controlled from 15~150 nm. Optimized CBHTFs exhibited ultra-low nonspecific protein adsorption below 5 ng/cm^2, and their 3D architecture allowed antibody loading to reach 693 ng/cm^2. This facile coating technique was also demonstrated to be directly applicable to an alternative hydrophilic substrate modified with SiO_2, indicating that its potentials for broad diagnostic applications.

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Table 1. Selection of hydrophilic SAM modifications on gold SPR chips

		SAM on gold chip		
	Bare gold	$C_{11}OH$	$C_{11}COOH$	C_{11}
Contact Angle (^{o})	25.6 ± 2.2	15.7 ± 1.3	5.2 ± 1.9	78.3 ± 3.6
Gel Thickness (nm)	22.3 ± 3.5	26.1 ± 2.2	29.7 ± 1.7	1.2 ± 0.8
Serum Fouling (ng/cm^2)	17.8 ± 4.3	9.4 ± 3.7	3.5 ± 1.3	–

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Figure Captions

Scheme 1. Overview of the coating process to generate CBHTFs on the surface of SPR chips.

Scheme 2. Chemical synthesis of carboxybetaine diacrylamide (CBAAX). (a) DIEA, Acryloyl chloride, DCM, 75% (b) $BrCH_2CO_2tBu$, CH_3CN, 60^oC, 73% (c) TFA:DCM 1:1, (d) MeOH, IRN-78 resin hydrolysis 84%.

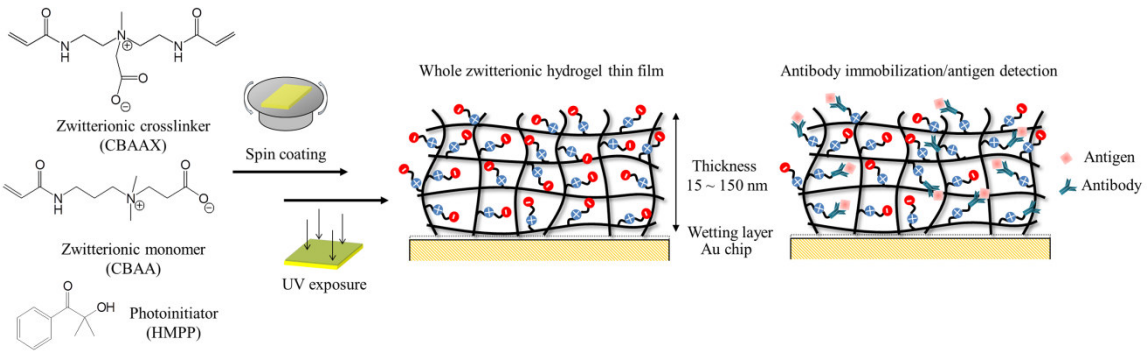
Fig 1. (a) Thickness of CBHTFs as a function of CBAAX ratio, which is presented as a molar percentage of the CBAA monomer; (b) thickness of CBHTFs in a PBS solution as a function of immersion time over 30 days. The error bars correspond to the standard deviation of three replicates.

Fig 2. Protein adsorption on CBHTFs from 100% human blood serum measured by a SPR sensor as a function of film thickness. The error bars correspond to the standard deviation of three replicates.

Fig 3. Typical SPR sensorgram for fouling tests on a CBHTF (thickness = 27.2 nm) modified SiO_2 surface in contact with undiluted human serum.

Fig 4. Typical SPR sensorgram for anti-TSH immobilization on CBHTF, followed by TSH antigen detection.

Fig 5. Antibody loading, antigen response, and nonfouling properties of CBHTFs as a function of CBAAX crosslinker ratio. The error bars correspond to the standard deviation of three replicates.



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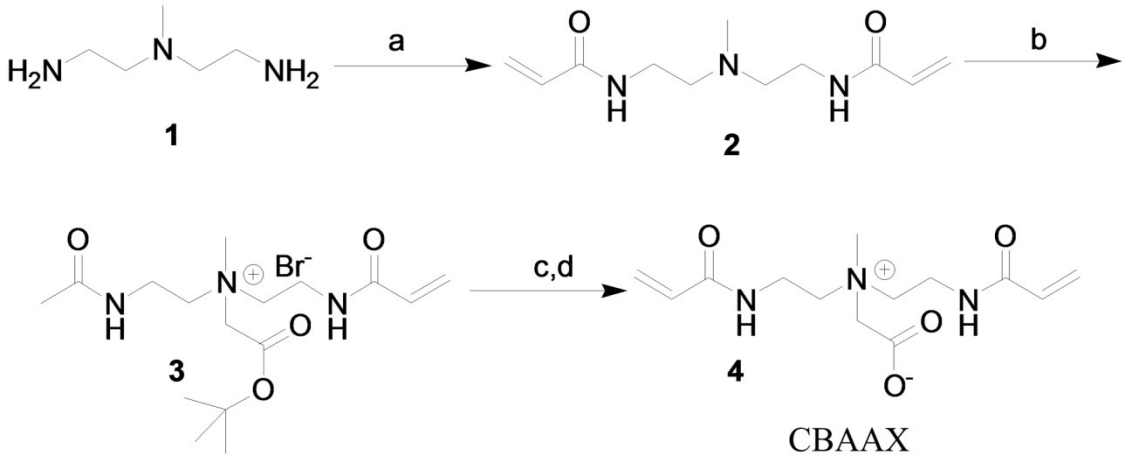

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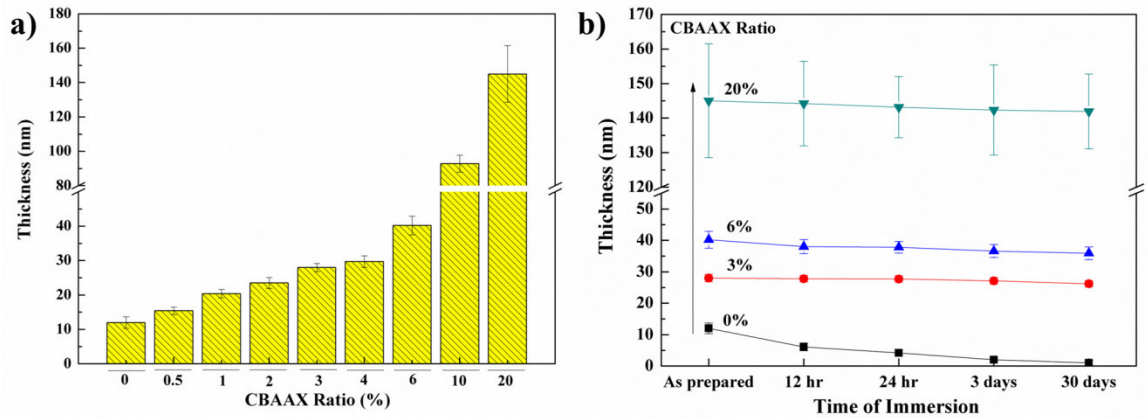

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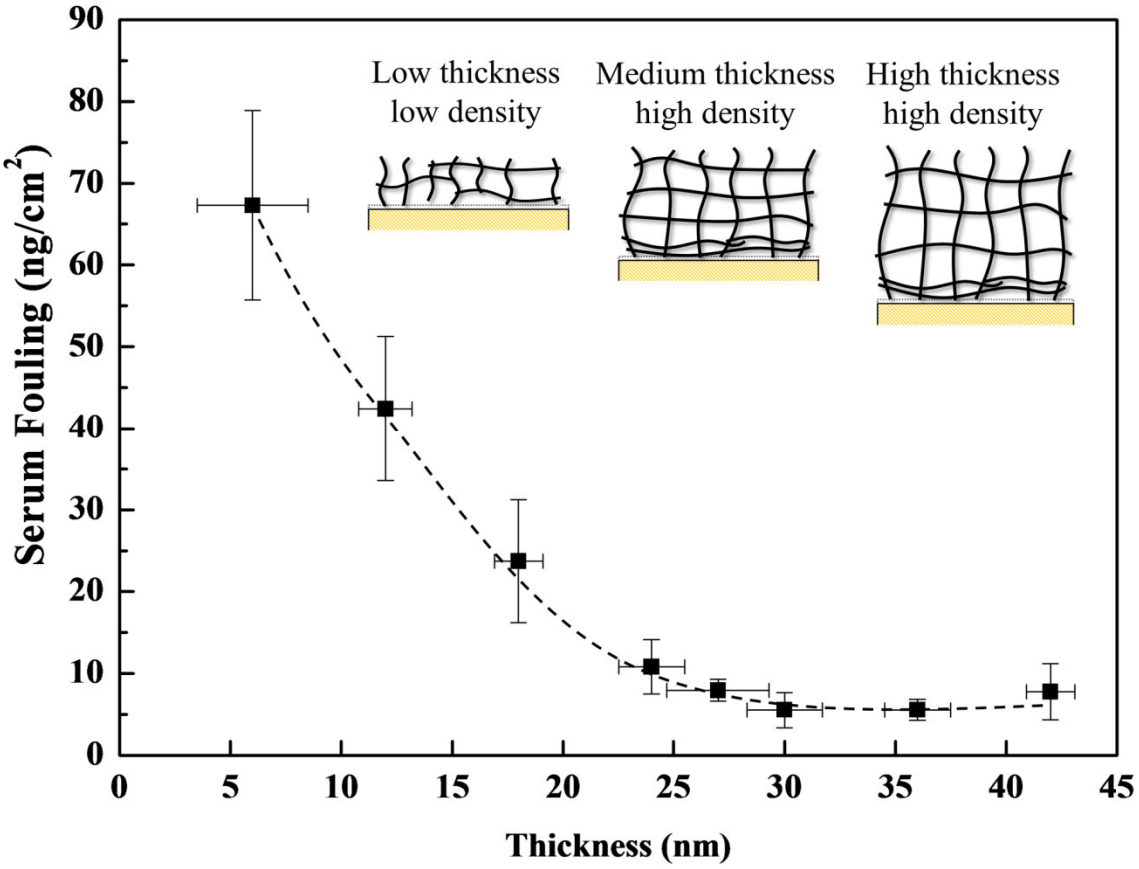

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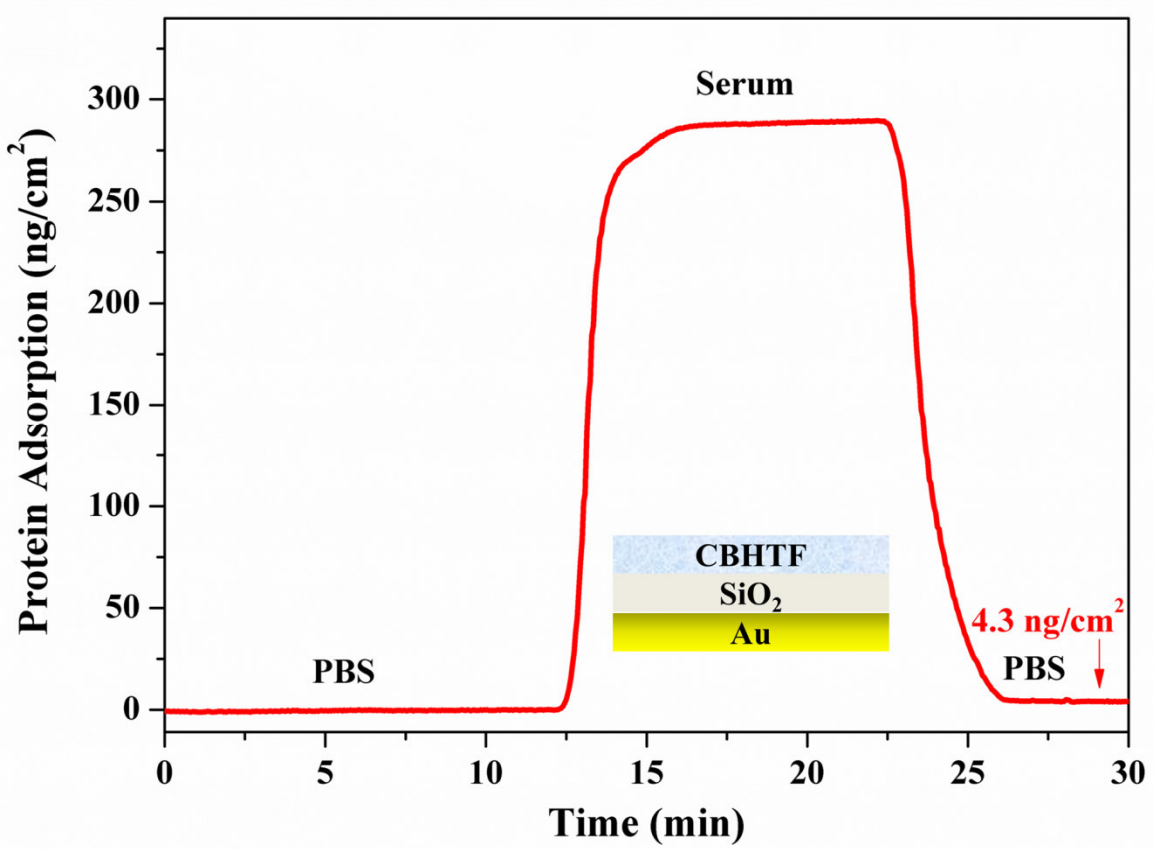

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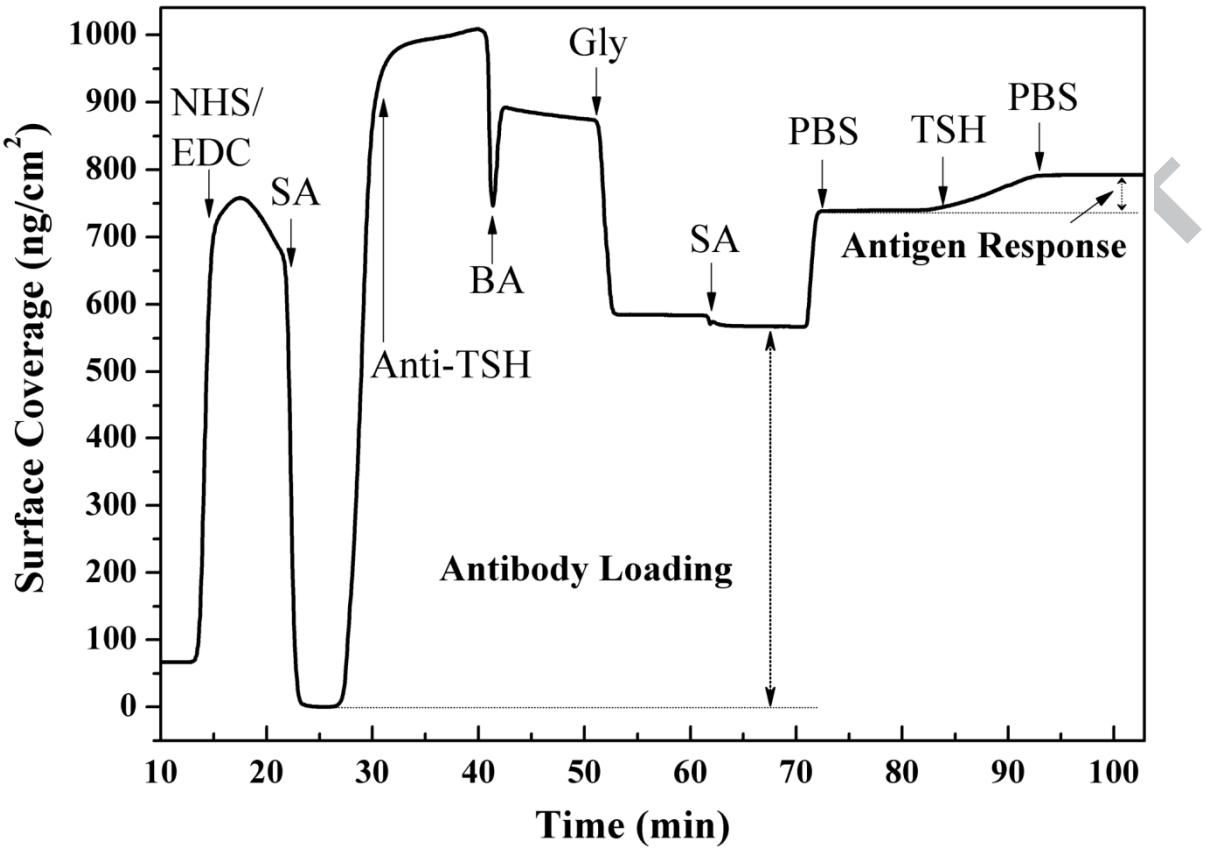

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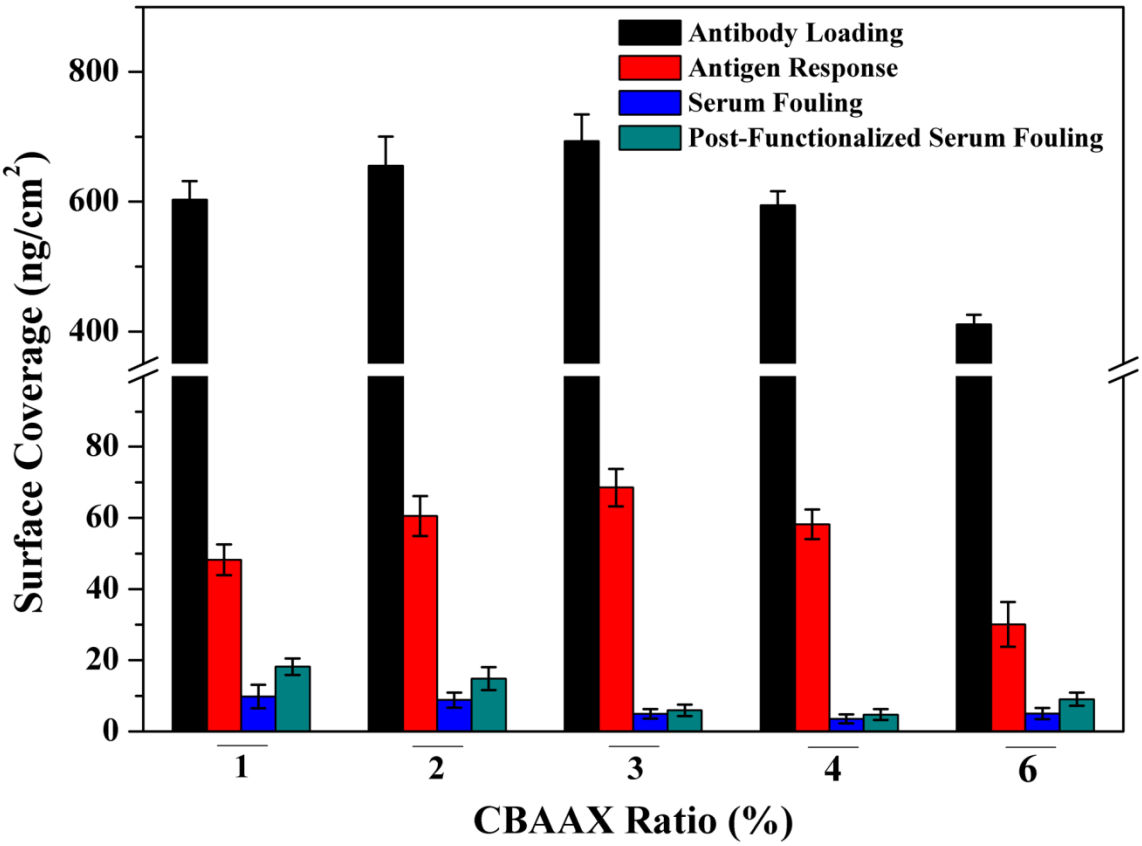

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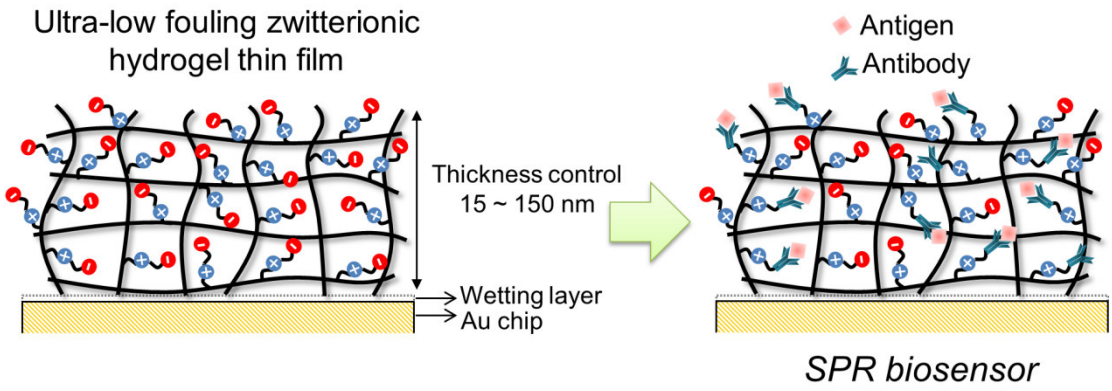
Graphical Abstract



Statement of significance

In this work, we developed an approach to realize ultra-low fouling and high ligand loading with a highly-crosslinked, purely zwitterionic, carboxybetaine thin film hydrogel (CBHTF) coating platform. The CBHTF on a hydrophilic surface demonstrated long-term stability. By varying the crosslinker content in the spin-coated hydrogel solution, the thickness of CBHTFs could be precisely controlled. Optimized CBHTFs exhibited ultra-low nonspecific protein adsorption below 5 ng/cm^2 measured by a surface plasmon resonance (SPR) sensor, and their 3D architecture allowed antibody loading to reach 693 ng/cm^2. This strategy provides a facile method to modify SPR biosensor chips with an advanced nonfouling material, and can be potentially expanded to a variety of implantable medical devices and diagnostic biosensors.