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# Photoiniferter-Mediated Polymerization of Zwitterionic Carboxybetaine Monomers for Low-Fouling and Functionalizable Surface Coatings

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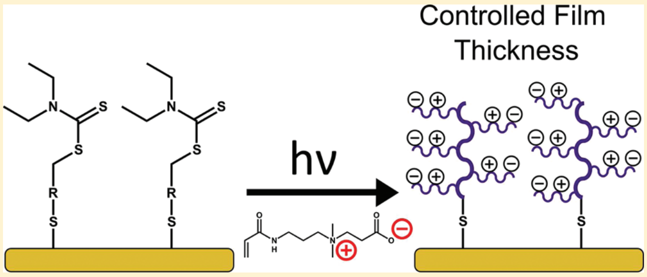
S *Supporting Information*

**ABSTRACT:** Surface-initiated photoiniferter-mediated polymerization (SI-PIMP) offers great promise for many applications primarily due to it lacking the requirement of a toxic catalyst as well as not being limited to specific types of monomers. In this work, zwitterionic carboxybetaine (pCB) polymer thin films with uniform and controlled thicknesses at high surface packing densities were achieved via SI-PIMP. It was found that the polymerization proceeded linearly with both UV-irradiation time and monomer concentration. Subsequent studies using a surface plasmon resonance biosensor showed pCB films to be ultralow fouling to undiluted human blood plasma. Antibody immobilization combined with excellent postfunctionalized protein resistance enabled the detection of an antigen down to 1 ng/mL from undiluted human plasma. These results demonstrate SI-PIMP as an attractive alternative to other commonly used polymerization techniques for zwitterionic monomers, such as atom transfer radical polymerization.



## 1. INTRODUCTION

Interfacial processes play a vital role in determining the fate of materials in numerous biomedical applications.[1,2] For example, the primary cause of failure for implantable medical devices is due to the initial adsorption of proteins followed by subsequent foreign body response mechanisms.[3−5] The physical and chemical properties of surfaces also affect *in vivo* nanoparticle therapeutics and diagnostics as the adsorption of blood proteins leads to their recognition and rapid clearance from the body, resulting in reduced circulation time and poor targeting efficiency.[4,6,7] Nonspecific protein binding also causes significant background noise for biosensors which reduces assay sensitivity.[8] This issue of biofouling has led to the necessity for developing surface grafted polymer brushes which can greatly increase interfacial biocompatibility.[1,2]

The most widely used low-fouling materials are based on poly(ethylene glycol) (PEG) and its derivatives.[9] However, PEG is a polyether that is susceptible to oxidative damage and enzymatic degradation, making it unsuitable in most long-term biomedical applications.[10,11] Furthermore, PEG has reduced functionality in the presence of undiluted complex media (e.g., human serum and plasma) and also requires complex chemical reactions to immobilize biomolecules.[12,13] Zwitterionic polymer films composed of carboxybetaine (pCB) and sulfobetaine (pSB) have been shown to be highly resistant to nonspecific protein adsorption and cellular adhesion all while maintaining excellent stability.[1,14] Ultralow fouling properties (defined as less than 5 ng/cm$^2$ of protein adsorption)[4] of these zwitterionic materials have been previously achieved from undiluted human plasma and serum.[13,14] Simulation studies have indicated that this resistance is due to electrostatically induced hydration, which is stronger than the hydrogen-bonding-induced hydration of PEG.[15] This provides the primary physical mechanism for improved protein resistance. Additionally, pCB films contain free carboxylic acid moieties which can be reacted with primary amines on biomolecules via common amino-coupling chemistry, yielding functionalized surfaces which maintain excellent nonfouling properties.[13,14] This enables the immobilization of biomolecules which can be used for enhancing drug targeting, tissue integration, and immobilization of antibodies for *in vitro* diagnostics, among many other applications, making pCB polymeric films highly desirable.

Ultralow fouling pCB substrates have primarily been obtained using surface-initiated atom transfer radical polymerization (SI-ATRP).[1,13,14] ATRP is one of the most widely used living radical polymerization methods and enables high density films with finely tuned thicknesses. These properties are vital for obtaining excellent nonfouling properties to undiluted complex media.[14] While ATRP may be an attractive choice for its robustness and simplistic components, there are certain limitations. First, the polymerization requires a transition metal catalyst for the reaction to take place. These materials are toxic and must be removed prior to use in biomedical applications. Second,

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the polymerization becomes difficult when using acidic monomers that can react or complex with the transition metal catalyst, limiting the otherwise wide range of compatible monomers.[16,17] Photopolymerization has recently emerged as an attractive alternative for preparing surface-initiated polymer brushes.[18,19] Surface-initiated polymerization (SIP) by photoirradiation is convenient due to the ability to control light exposure spatially and temporally, thereby enabling a versatile route to multidimensionality in two and three dimensions without being limited to specific types of monomers. This approach also enables much larger surfaces to be more easily coated compared to ATRP. A key advantage of this SIP is that it relies only on a UV-light source for the reaction to take place, thereby eliminating the need for removing toxic catalyst complexes which affect the biocompatibility of resulting polymeric films.[16] However, the majority of surface-initiated photopolymerization reactions are based on conventional free radical mechanisms which provide only very limited control over the film thickness and polymerization kinetics.[2]

In order to obtain excellent protein resistance from complex media, it is essential to first gain control over the polymerization reaction thereby enabling the formation of uniform surface coatings of a desired thickness. Such control can be accomplished by maintaining the equilibrium between activated propagating chains and chains that have been capped by a deactivating complex. In UV-irradiated reactions, this is achieved with the use of a photoiniferter that acts as an initiator, chain transfer agent, and terminator.[20−22] Photoiniferters contrast with simple photoinitiators, which are more commonly employed in photopolymerization procedures, as the latter generates free radicals with an extremely high reactivity[2] and thus significantly limits the ability to obtain uniform and controlled polymeric films. Solution-based polymerizations using small photoiniferters have been shown to yield much lower polydispersity indices as compared to those prepared via a simple photoinitiator.[23] This indicates the inclusion of reversible termination events, thereby implying a "quasi-living" mechanism of growth for the formation of uniform polymer chains.[24]

In contrast to solution polymerization, surface-initiated polymerization methods present the unique challenge of creating dense polymer brushes grown from a substrate. Previous studies using surface-initiated photoiniferter-mediated polymerization (SI-PIMP) of methacrylic acid and *N*-isopropylacrylamide (NIPAM) from gold-coated self-assembled monolayers (SAMs) of dithiocarbamate iniferters revealed that it was possible to precisely control the film thickness based on irradiation time.[25,26] While SI-PIMP has been demonstrated with neutral (nonionic), anionic, and cationic monomers for making surfaces ranging from a simple coating to block copolymers to gradient microarchitectures,[2,16] there have been no reports of zwitterionic compounds. Zwitterionic polymers are unique compared to simple polyelectrolytes as they exhibit the antipolyelectrolyte effect. Recently, a disulfide-terminated photoiniferter was used with zwitterionic monomers to synthesize polymer chains *in* solution which were then attached to a gold surface via the disulfide bond. With this "graft to" method, limited reduction in single protein adsorption was observed.[27] These studies suggest the possibility for combining SI-PIMP with zwitterionic CB monomers to create highly biocompatible polymeric films especially in the challenging context of undiluted complex media.

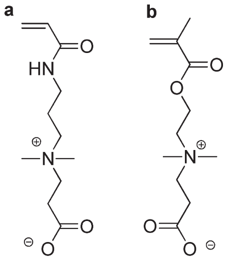


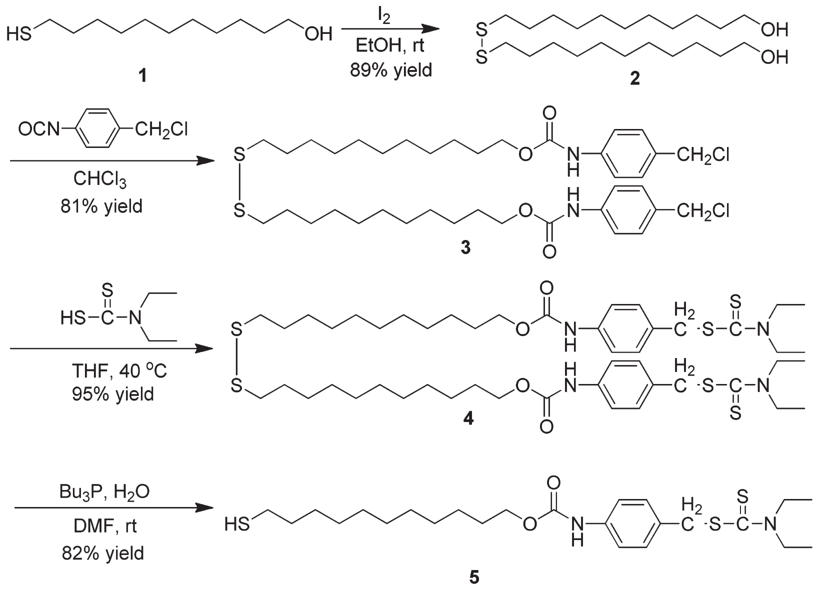
**Figure 1.** Chemical structures of zwitterionic CB monomers: (a) carboxybetaine acrylamide (CBAA) and (b) carboxybetaine methacrylate (CBMA).

Here, for the first time, the protein-resistant and functionalizable properties of zwitterionic carboxybetaine monomers were combined with the advantageous SI-PIMP approach to create uniform polymer films on gold substrates. The polymerization conditions necessary to precisely control the film thickness, enabled by the "quasi-livingness" of this approach, for both carboxybetaine acrylamide (CBAA, Figure 1a) and carboxybetaine methacrylate (CBMA, Figure 1b) were studied. Using a surface plasmon resonance (SPR) biosensor, the resulting thin films of pCBAA demonstrated ultralow fouling properties and highly specific detection from undiluted human blood plasma.

## 2. EXPERIMENTAL SECTION

**Materials.** Tetrahydrofuran (THF) was purchased from OmniSolv (Salisbury, NC). Ethanol (200 proof) was purchased from Decon Laboratories, Inc. (King of Prussia, PA). Phosphate-buffered saline (PBS, 0.01 M phosphate, 0.138 M sodium chloride, 0.0027 M potassium chloride, pH 7.4), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 11-mercaptoundecan-1-ol, 4-(chloromethyl)phenyl isocyanate, dibutyltin dilaurate, diethylammonium salt of diethyldithiocarbamic acid, 2-(*N*,*N*′-dimethylamino)ethyl methacrylate (DMAEMA, 98%), triethylamine, anhydrous acetone, methanol, and tributylphosphine were purchased from Sigma-Aldrich (St. Louis, MO). Sodium carbonate anhydrous was purchased from EMD Chemicals (Darmstadt, Germany). Sodium chloride (NaCl) and ether were purchased from J.T. Baker (Phillipsburg, NJ). Sodium acetate anhydrous was purchased from Fluka (subsidiary of Sigma-Aldrich, St. Louis, MO). 1-Ethyl-3-(3-(dimethylamino)-propyl)carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) were purchased from Acros Organics (Geel, Belgium). $\beta$-Propiolactone was purchased from Alfa Aesar (Ward Hill, MA). *N*-[3-(Dimethylamino)propyl]acrylamide (DMAPA, 98%) was purchased from TCI America (Portland, OR). Pooled human blood plasma was purchased from BioChemed Services (Winchester, VA). Antibody to thyroid stimulating hormone (anti-TSH) and the TSH antigen were purchased from ThermoFisher Scientific (Waltham, MA). The water used in these experiments was purified using a Millipore water purification system with a minimum resistivity of 18.2 M$\Omega$ cm.

**Synthesis of Dithiocarbamate Photoiniferter.** The synthesis of photoiniferter **5**, 11-mercaptorundecane-1-[4({[(diethylamino)-carbonothioyl]thioethyl}phenyl)carbamate], is shown in Scheme 1. 11,11′-Dithiodiundecan-1-ol (**2**), dithiodiundecane-11,1-diylbis{[4-(chloromethyl)phenyl]carbamate} (**3**), and dithiodiundecane-11,1-diylbis-[4({[(diethylamino)carbonothioyl]thioethyl)phenyl]carbamate} (**4**) were prepared following the procedures reported previously.[26] After dissolving compound **4** (3.36 g, 3.48 mmol) in DMF (30 mL), Bu$_3$P (1.3 mL, 5.22 mmol) and water (0.6 mL) were added to the reactor. The mixture was stirred at room temperature under $N_2$ atmosphere for 16 h followed by solvent removal with a rotary evaporator. The residue was purified by column chromatography (silica gel, eluent: hexanes/ethyl acetate 5:1) to provide 2.81 g of product **5** as a white powder in 83% yield. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 7.34 (s, 4H), 6.59 (br, 1H), 4.52 (s, 2H), 4.17 (t, $J$ = 6.6 Hz, 2H), 4.05 (q, $J$ = 6.6 Hz, 2H), 3.74 (q, $J$ = 6.9 Hz, Scheme 1. Chemical Synthesis of Thiol-Based Dithiocarbamate Photoiniferter 5



2H), 2.54 (q, $J$ = 7.2 Hz, 2H), 1.75−1.56 (m, 4H), 1.48−1.32 ppm (m, 20H).

**Synthesis of Carboxybetaine Acrylamide (CBAA) Monomer.** (3-Acryloylaminopropyl)-(2-carboxyethyl)dimethylammonium (CBAA) was synthesized by reacting 48 mL of DMAPA with 25 g of $\beta$-propiolactone in 400 mL of anhydrous acetone at 0 °C under nitrogen protection. After removing the ice bath at 20 min, the solution was allowed to warm up to room temperature. After 6 h, the product was filtered, washed with ether, and dried under vacuum. The rough product, a white precipitate, was redissolved in a 30% (v/v) triethylamine in methanol solution and stirred overnight. After concentrating the solution, the CBAA was precipitated with acetone and then filtered. The solids were suspended in acetone and ether, for 1 h each, dried under vacuum, and then stored at 4 °C. Yield: 61%.$^1$H NMR (Bruker 500 MHz, DMSO-$d_6$): 8.61 (t, 1H,N−H), 6.28 (t, 1H, CHH=CH), 6.13 (t, 1H, CHH=CH), 5.61 (t, 1H, CHH=CH), 3.44 (t, 2H, N−$CH_2$−$CH_2$−COO), 3.21 (m, 4H, NH−$CH_2$−$CH_2$−$CH_2$), 2.97 (s, 6H, N−$(CH_3)_2$), 2.25 (t, 2H, $CH_2$−COO), 1.87 (t, 2H, NH−$CH_2$−$CH_2$−$CH_2$).

**Synthesis of Carboxybetaine Methacrylate (CBMA) Monomer.** 2-Carboxy-*N*,*N*-dimethyl-*N*-(2′-(methacryloyloxy)ethyl)ethanaminium inner salt (CBMA) was synthesized by the reaction of 60 mL of DMAEMA and 25 g of $\beta$-propiolactone in 300 mL of anhydrous acetone. The reaction mixture was stirred under nitrogen protection at 0 °C for ~2 h. The white precipitate was filtered, washed with ether, and dried under reduced pressure to obtain the final CBMA monomer which was stored at 4 °C. Yield: 60%. NMR was recorded on a Bruker spectrometer using deuterated water as solvent. $^1$H NMR (300 MHz): $\delta$ 6.06 (s, 1H, =CH), 5.68 (s, 1H, =CH), 4.55 (t, 2H, $OCH_2$), 3.70 (t, 2H, $CH_2N$), 3.59 (t, 2H, $NCH_2$), 3.10 (s, 6H, $NCH_3$), 2.64 (t, 2H, $CH_2COO$), 1.84 (s, 3H, $=CCH_3$). $^{13}$C NMR (500 MHz): $\delta$ 180.27, 172.43, 139.16, 131.77, 66.38, 66.31, 62.44, 55.35, 34.76, 21.30.

**SPR Sensor, Chips, and Calibration of the Surface Sensitivity.** A laboratory SPR sensor developed at the Institute of Photonics and Electronics, Prague, Czech Republic, was used as described previously.[8,13] This custom-built SPR is based on the attenuated total reflection method and wavelength modulation. It is equipped with a four-channel flow-cell and temperature control and uses a peristaltic pump for delivering samples. SPR sensor chips (2.0 × 2.5 cm) were made of a glass slide coated with an adhesion-promoting titanium film (~2 nm) followed by a gold film (~48 nm) using an electron beam evaporator.

Since the SPR sensitivity depends on the distance of the binding event from the SPR active surface, the sensor response due to the polymer films had to be calibrated.[8] This was done using previously described methods.[13,14] The ~26 nm pCB film in this work resulted in a calibration factor of 1.31. Hence, a 1 nm shift in the resonant wavelength corresponded to a change in protein surface coverage of ~22.3 ng/cm$^2$.[28]

**Preparation of Photoiniferter-Coated SAMs on SPR Chips.** Prior to the formation of photoiniferter SAMs on gold-coated SPR chips, the bare substrates were washed with water and ethanol, cleaned with a UV-ozone cleaner for 20 min, washed with water and ethanol, and then dried under a stream of filtered air. The photoiniferter SAMs were then formed by soaking the cleaned gold-coated substrates in THF containing 0.2 mM photoiniferter (Scheme 1, compound **5**) at room temperature for 24 h. Before polymerization, the substrates were rinsed with THF, placed in pure THF for 1−2 min, and then dried with a stream of filtered air.

**Surface-Initiated Photoiniferter-Mediated Polymerization (SI-PIMP).** The monomer solution was first prepared by weighing out the appropriate monomer and adding solvents to achieve the desired concentration and solvent composition. The solution was stirred with a magnetic stir bar for 1 h and then deoxygenated by passing a continuous stream of nitrogen through the liquid for 15 min. The substrate prepared with the photoiniferter SAM was placed in a reaction tube manufactured from rectangular quartz tubing. The test tube was sealed with a rubber septum and placed under nitrogen protection. The $N_2$ purged monomer solution was then transferred to the reaction tube using a syringe under nitrogen protection. A stream of nitrogen was allowed to pass through the monomer solution in the reaction tube for 1−2 min before being removed and wrapped with parafilm. In order to prevent the cleavage of the thiol−gold bond of the photoiniferter SAM, a 280 nm cutoff filter was mounted to the outside of the reaction tube. Samples were then irradiated with 302 nm-centered UV light (UVP, model UVM-57) for the desired reaction time. Following the polymerization, the substrates were removed from the reactor, washed with PBS, and then stored in PBS overnight.

**Measurements of Film Thickness by Ellipsometry.** Dry film thickness measurements were made using a multiwavelength ellipsometer (J.A. Woollam Co., Inc., Model alpha-SE). Analysis was performed using a Cauchy model with a bare SPR substrate.

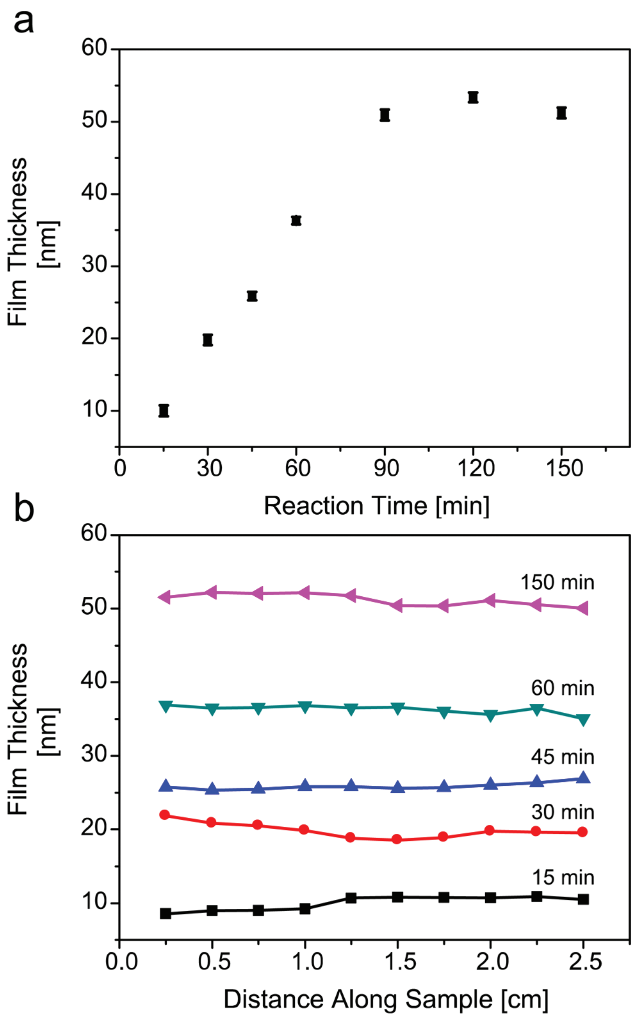


**Figure 2.** Thickness of CBMA polymer films made via SI-PIMP as a function of UV-irradiation time. Using a 0.3 M monomer concentration and pure water as the solvent, (a) represents the average film thickness of each sample and (b) shows the localized measurements along the length of each substrate. These results indicate the ability to obtain uniform and controlled polymeric films of a desired thickness simply by controlling the UV-irradiation time. The gold-coated substrate is approximately 2.0 × 2.5 cm.

**Measurements of Nonspecific Protein Adsorption by SPR.** The nonspecific protein adsorption of the pCB polymer films formed via SI-PIMP was determined with a SPR biosensor using a flow rate of 40 $\mu$L/min at 25 °C. After first establishing a baseline using PBS, undiluted human plasma was flowed for 10 min, followed by buffer to reestablish the baseline. Protein adsorption was quantified as the difference between buffer baselines and converted to a surface coverage.

**In Situ Functionalization of pCB Polymer Surfaces.** The functionalization procedure was monitored step-by-step in real time using a SPR sensor at 25 °C. Sodium acetate buffer (10 mM) at pH 5.0 (SA) was first injected at 30 $\mu$L/min to obtain a stable baseline. The carboxylic acid groups of the polymer surface were then activated by flowing a freshly prepared solution of 0.05 M NHS and 0.2 M EDC in water for 7 min. Following a brief injection of SA buffer, a solution of anti-TSH (50 $\mu$g/mL) in HEPES buffer (pH 7.5) was flowed over the activated surface for 20 min at 20 $\mu$L/min. Subsequent washing of the functionalized surface for 10 min with 10 mM sodium carbonate (pH 10) containing 0.3 M NaCl (SC) at 30 $\mu$L/min removed noncovalently bound ligands and deactivated residual NHS esters. SA buffer was then used to reestablish a stable baseline. The amount of immobilized antibodies was determined as the different between the SA injection following EDC/NHS activation and the final baseline.

**Measurements of Postfunctionalized Nonfouling and Specific Protein Detection.** Following antibody immobilization, the pCBAA surface was washed with PBS buffer until a steady baseline was established at 50 $\mu$L/min and 25 °C. For postfunctionalized nonfouling, undiluted human blood plasma was then injected for 10 min followed by PBS for an additional 10 min. The net adsorption was calculated as the difference between buffer baselines and converted to a surface coverage. For antigen detection, anti-TSH was immobilized onto all four sensor channels using the above protocol. After establishing a stable baseline with PBS, samples of TSH spiked 100% human plasma at six concentrations (1, 10, 40, 100, 200, and 1000 ng/mL) were then flowed through three channels, and unspiked plasma was flowed through the fourth channel for 10 min followed by PBS. The amount of antigen binding was calculated as the difference between the spiked buffer baselines relative to that of the unspiked channel. All analyte concentrations were tested in triplicate except for 10 and 100 ng/mL, which were done in duplicate.

## 3. RESULTS AND DISCUSSION

**CBMA Polymer Films by SI-PIMP.** SI-PIMP has a great potential for achieving polymer films with uniform and controlled thicknesses.[25] In order to obtain this goal, appropriate polymerization conditions were first established. The relationship between film thickness and duration of UV exposure (up to 150 min) for the polymerization of CBMA is presented in Figure 2a using a monomer concentration of 0.3 M in pure water.

The observed linearity of film growth as a function of UV-irradiation time (up to 90 min) showed that the surface polymerization proceeded via the expected "quasi-living" mechanism with relatively consistent growth of polymer chains across the substrate.[2] It also enables a desired thickness to be easily obtained by simply adjusting the exposure time. However, the nonlinearity associated with the leveling off of the curve at ~90 min (~51 nm in thickness) is likely due to chain−chain termination reactions. Such irreversible events occur as a result of an insufficient concentration of deactivating dithiocarbamyl radicals near the surface to maintain the polymerization equilibrium over the duration of the reaction.[22] While some reports of SI-PIMP in the literature have not observed such phenomena,[25] the extent of these termination events as well as the time and thickness at which they occur is highly dependent upon numerous factors including the specific monomer used, monomer concentration, solvent, light intensity, reaction time, and the initiator surface concentration prior to polymerization.

Another observation of Figure 2a is the lack of an apparent induction time during which there is a slow initial thickening rate (i.e., a slow initial increase in polymer thickness at the very beginning of the polymerization) followed by an acceleration of growth to yield linear behavior. The results here show that the linear "quasi-living" behavior began almost instantly. Previous studies have suggested that this induction period is caused by the transition between different configurations of surface tethered chains, a change from "mushroom" to extended "brush" configurations as the film grows.[2] This indicates that pCB films made via SI-PIMP evolve very rapidly into uniformly extended chains. However, the time at which this transition occurs has been shown to vary significantly, from a few seconds to hours and is also

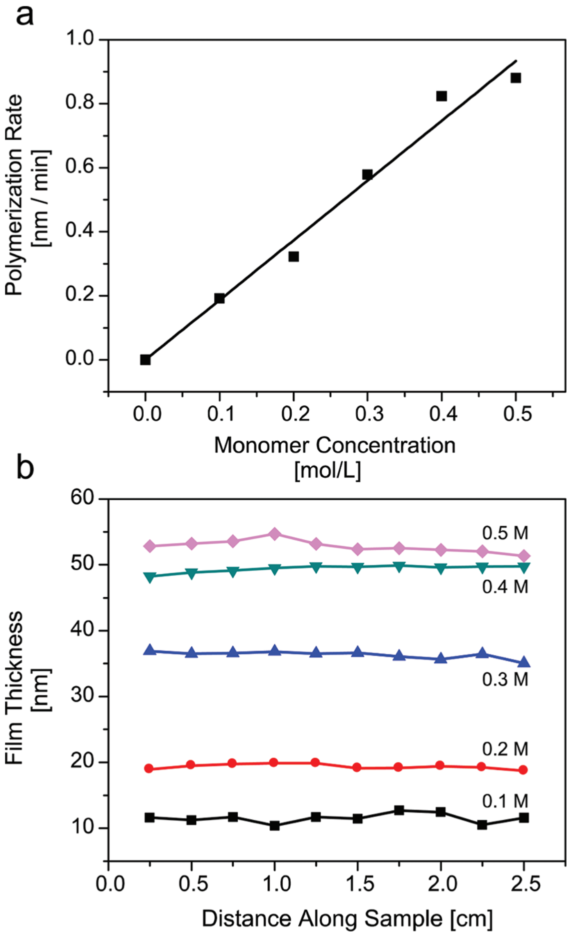


**Figure 3.** (a) SI-PIMP polymerization rate of CBMA as a function of monomer concentration using an irradiation time of 60 min and a pure water solvent. (b) Localized film thickness measurements along the length of each sample at the end of the reaction for each corresponding monomer concentration. These results demonstrate the "quasi-living" behavior of zwitterionic monomers as well as the ability to obtain uniform and controlled polymer thicknesses. The gold-coated substrate is approximately 2.0 × 2.5 cm.

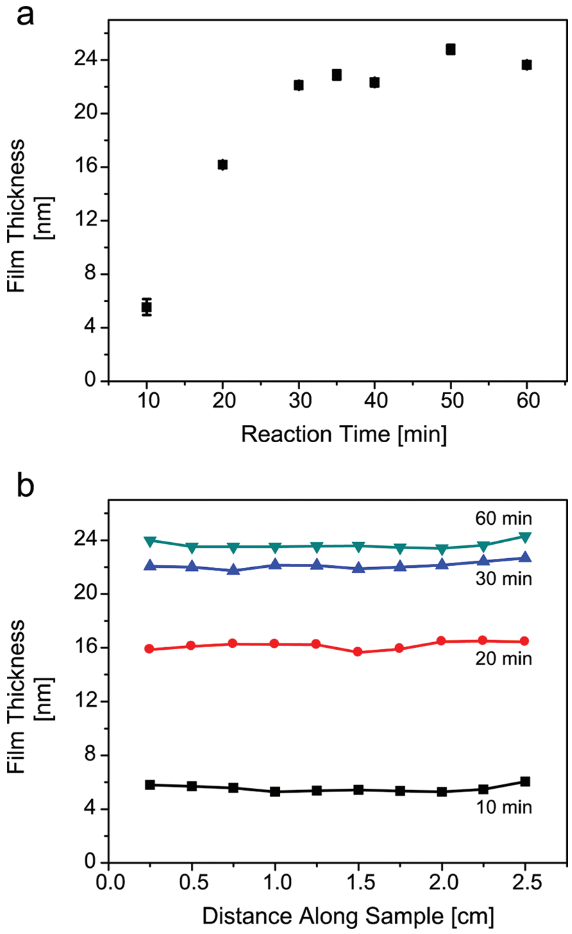


**Figure 4.** Polymer film thickness as a function of UV-irradiation time for the SI-PIMP of CBAA using a monomer concentration of 0.15 M in a (4:1) mixture of methanol/water. (a) The average film thickness and (b) localized measurements along the length of each sample. The gold-coated substrate is approximately 2.0 × 2.5 cm.

dependent upon the experimental variables mentioned previously, which may explain why such phenomena was not observed in this work.

The localized film thickness values along the length of each sample (2.5 cm) are shown in Figure 2b. These values were averaged to provide the values plotted in Figure 2a. Minimal variation along each sample demonstrates a uniform polymer brush was formed, which has been found to be essential for achieving desirable ultralow fouling properties.[4]

Monomer concentration is also a convenient parameter to vary in order to achieve a desired film thickness. A reduction in consumption of the reactive species is especially important for those systems in which the monomer is not readily available and is thus a factor for the potential scalability of the SI-PIMP approach. Using a fixed irradiation time of 60 min and pure water as the solvent, the effect of CBMA monomer concentration on polymerization rate was also studied. As shown in Figure 3a, a linear trend was obtained, with all polymer films exhibiting uniform localized thicknesses (Figure 3b). Furthermore, these results indicated the potential for obtaining even thicker films at higher concentrations if such surfaces are desired for an intended application. The linearity of the polymerization rate as a function of monomer concentration indicates first-order kinetics, which would be expected for a controlled SI-PIMP reaction, and is further evidence that zwitterionic monomers exhibit "quasi-living" behavior.[2] Taken together, Figures 2 and 3 demonstrate that both exposure time and monomer concentration can be used to adjust the final film thickness of zwitterionic CBMA surface coatings.

**CBAA Polymer Films by SI-PIMP.** Prior to determining the protein-resistant properties of carboxybetaine polymer films prepared by SI-PIMP, the versatility of the approach for other zwitterionic monomers was investigated. Figure 4a shows the relationship between film thickness and irradiation time for SI-PIMP of CBAA using a monomer concentration of 0.15 M in a (4:1) mixture of methanol/water with accompanying localized measurements (Figure 4b). The results (Figure 4a) revealed a very similar trend to that obtained for the polymerization of CBMA (Figure 2a). Each consisted of a linear region, a flattened region at longer reaction times, indicating chain—chain termination events, and the lack of an induction period, despite different monomer backbones with inherently different reactivities and solubilities.

Table 1. Nonfouling and Functionalization Properties of pCBAA Films Prepared by SI-PIMP Using 0.2 M CBAA in a 15% (v/v) Water/Methanol Solvent and a 15 min UV-Irradiation Time

| film thickness (nm) | plasma fouling (ng/cm$^2$) | postfunctionalization plasma fouling (ng/cm$^2$) | anti-TSH immobilization (ng/cm$^2$) |
|---|---|---|---|
| 25.6 ± 3.3 | 4.2 ± 0.3 | 8.8 ± 3.9 | 279 ± 40 |

However, the CBAA polymerization produced a shorter linear region with a maximum film thickness which was thinner than that for CBMA. In addition to the difference in reactivity between the two monomers, another possible explanation for this result is that the monomer concentration for the latter case was twice as high. Previous results have shown that the maximum film thickness increases with increasing monomer concentration.[22] In relating the SI-PIMP result observed here to ATRP, both being controlled radical polymerization methods, it has been shown that the equilibrium constants for methacrylate monomers are greater than that of acrylamides (i.e., the activation rate constant is larger).[29] This implies that it is more difficult to react with acrylamide monomers from a growing chain end, but once the radical is formed, it has much higher reactivity due to being less stable. The shorter linear region with a lower final thickness achieved for CBAA may be the result of these less stable propagating chain ends which could subsequently terminate faster. As mentioned previously and as with other "living" radical polymerizations, different monomers require individually optimized reaction conditions, thus making a direct comparison of parameters difficult. Regardless, these results show the ability of SI-PIMP to obtain a wide range of controlled and uniform film thicknesses for both carboxybetaine zwitterionic monomers.

**Ultralow Fouling and Functionalization Properties of pCB Films Prepared via SI-PIMP.** CBAA films were chosen to proceed with the evaluation of fouling and functionalization properties. Previous pCBAA films formed via SI-ATRP have indicated an optimal thickness of ~21 nm for obtaining the best protein resistant properties (i.e., <5 ng/cm$^2$ of nonspecific adsorption to undiluted human serum and plasma).[14] Therefore, in order to demonstrate the suitability of zwitterionic CB films made via SI-PIMP for biomedical applications, ~26 nm films were used. As determined with a SPR biosensor, the pCBAA-coated substrates achieved ultralow fouling levels from undiluted human plasma (Table 1). This indicates the ability of SI-PIMP to create high density, controlled, and uniform zwitterionic polymer brushes of a desired thickness, which are the key criteria necessary for achieving such fouling resistance. To put this into perspective, oligo-ethylene glycol SAMs on gold substrates enabled ~90 ng/cm$^2$ of nonspecific binding to 67% plasma.[30] As mentioned above, another attractive characteristic of carboxybetaine-based monomers is the presence of carboxylic acid groups which can be used to immobilize biomolecules via commonly used amino-coupling chemistry. The ability to achieve these properties was tested by functionalizing pCBAA surfaces with anti-TSH and determining the sensitivity and specificity of the subsequent surface for detecting TSH antigen spiked directly into undiluted human plasma. However, the pCBAA films prepared via SI-PIMP must also maintain excellent nonfouling properties following functionalization.

Table 1 shows the average anti-TSH immobilization and postfunctionalized fouling values for pCBAA films prepared under fixed reaction conditions (0.2 M CBAA in 15% (v/v) water/methanol solvent using a 15 min UV-irradiation time). A standard SPR sensor-gram representing the functionalization procedure is shown in the Supporting Information (Figure S1). The level of nonspecific binding from undiluted human blood plasma is still very low after functionalization of the film with an effective monolayer of antibody (~200−300 ng/cm$^2$).[4] While carboxylic group activation by EDC/NHS chemistry enables antibody immobilization, the deactivation of unreacted groups is necessary for the surface to return back into the original nonfouling background. Thus, a balance between surface activation and deactivation must be obtained in order to achieve sufficient antibody immobilization while maintaining excellent postfunctionalized nonfouling properties.[31] Since the functionalized surface was evaluated for its resistance to nonspecific protein adsorption from highly complex 100% human blood plasma, the results indicate that the nonfouling property was largely retained after antibody attachment. SPR sensorgrams demonstrating the nonfouling properties to undiluted plasma, before and after functionalization, are shown in the Supporting Information (Figure S2). As shown, the buffer baseline following plasma exposure for both surfaces (pCBAA and anti-TSH coated pCBAA) essentially

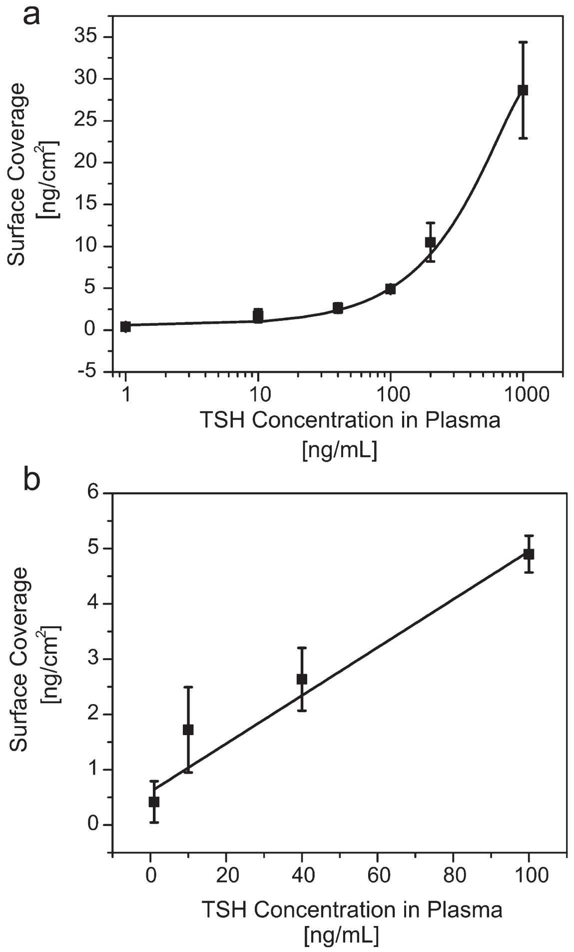


**Figure 5.** (a) Standard detection curve for TSH spiked into undiluted human blood plasma using anti-TSH-functionalized pCBAA films prepared via SI-PIMP. Antibody immobilization amounts were ~280 ng/cm$^2$, and final TSH concentrations ranged from 1 to 1000 ng/mL. (b) Linear region of the standard curve which indicates a limit of detection of ~1 ng/mL.

returned to a similar low level, indicating excellent protein resistance.

The standard curve for TSH detection from undiluted human plasma by antibody-functionalized pCBAA surfaces is shown in Figure 5a. Plasma samples were spiked with TSH to final concentrations of 1−1000 ng/mL. The linear region of the curve is plotted in Figure 5b, indicating a limit of detection (LOD) of ∼1 ng/mL. Previous results of antibody functionalized pCBAA surfaces prepared via SI-ATRP have achieved a LOD of ∼10 ng/mL.[13] However, these results cannot be directly compared as the two antigens have different molecular weights as well as different antibody affinity binding constants. As a reference, the LOD of TSH from buffer using an optical waveguide biosensor was found to be ∼10 ng/mL. This value was obtained using an EDC/NHS antibody functionalized silane coating (consisting of reactive acyl chloride end groups) which was blocked prior to antigen detection in order to minimize unwanted background noise.[32] The low level of nonspecific adsorption on pCBAA films, combined with their ability to effectively immobilize biomolecules while maintaining protein resistance, indicates the great potential of SI-PIMP with zwitterionic CB monomers for meeting the demanding requirements of biomedical applications.

## 4. CONCLUSIONS

Zwitterionic carboxybetaine polymeric films with uniform and controlled thicknesses at high surface packing densities were successfully achieved via SI-PIMP. The effect of UV exposure time and monomer concentration on polymerization was studied for two carboxybetaine compounds. The duration of UV exposure resulted in a linear increase in thickness until sufficient chain termination occurred, after which the surface tethered polymer film could no longer continue to grow. A simple linear increase in the polymerization rate was also observed when monomer concentration was varied. Taken together, these results confirmed the “quasi-living” nature of SI-PIMP with zwitterionic carboxybetaine monomers and also demonstrated the great potential for scalability offered with this approach. The ability to obtain uniform films with controlled thickness was critical for achieving excellent protein resistant properties from complex media.

As evaluated with a SPR biosensor, the protein-resistant properties of pCBAA films made by SI-PIMP were found to be ultralow fouling to undiluted human blood plasma. The immobilization of an effective monolayer of antibody followed by low postfunctionalized nonspecific adsorption enabled the sensitive and specific detection of the corresponding antigen directly from undiluted plasma down to ∼1 ng/mL. These results show the great potential of SI-PIMP with zwitterionic carboxybetaine monomers to enable surface coatings for numerous biomedical applications including medical diagnostics and targeted drug delivery.

## ■ ASSOCIATED CONTENT

**Ⓢ Supporting Information.** Figures S1 and S2. This material is available free of charge via the Internet at http://pubs.acs.org.

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