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Nonfouling polyampholyte polymer brushes with protein conjugation capacity

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ABSTRACT

The elimination of nonspecific protein adsorption is an important challenge in many biomaterial applications. To address this issue, numerous nonfouling chemistries have been investigated. This work reports on the dual functional properties of a polyampholyte copolymer composed of positively charged [2-(acryloyloxy) ethyl] trimethyl ammonium chloride (TMA) and negatively charged 2-carboxy ethyl acrylate (CAA) monomers. TMA:CAA copolymers have previously been shown to have nonfouling properties, but the optimal conditions for nonfouling have not been determined. To accomplish this, the thickness of the polymer brush coating was varied by manipulating the surface-initiated atom transfer radical polymerization conditions. The nonspecific adsorption of fibrinogen and lysozyme was measured as a function of the copolymer brush thickness using a surface plasmon resonance biosensor. At the optimal thickness for nonfouling, nonspecific adsorption from 10% and 100% fetal bovine serum (FBS) was determined. The results indicate that at the optimal copolymer brush thickness, TMA:CAA polyampholyte materials have ultralow fouling characteristics even upon exposure to 100% FBS. The dual functional properties of TMA:CAA copolymers were demonstrated by conjugating fibrinogen to the copolymer brush over a range of brush thicknesses. The conjugation experiments clearly demonstrate that TMA:CAA copolymers have the capacity for protein conjugation at the optimal thickness for nonfouling. However, the conformational state of the copolymer brush chains impacts the overall conjugation capacity of the system. The results of this investigation indicate that TMA:CAA polyampholyte surfaces show promise for biosensor and biomaterial applications where their dual functional properties would be beneficial.

1. Introduction

Nonfouling materials have widespread applications including biosensors, drug delivery, and biomaterials [1]. Therefore, there is significant interest in the development of novel approaches for eliminating nonspecific protein adsorption. Poly(ethylene glycol) (PEG) and oligo(ethylene glycol) (OEG) have been extensively studied for their use as nonfouling surfaces [2–5]. The nonfouling properties of PEG have been attributed to the formation of a strong hydrogen-bonded hydration layer, that limits protein interactions with the underlying PEG materials [6]. However, it has been suggested that PEG decomposes in the presence of transition metal ions and oxygen, which are present in biochemically relevant solutions [7,8]. This has led to the search for alternatives to PEG.

Recent research has shown that zwitterionic materials represent a promising alternative to PEG-based materials [9]. Phosphorylcholine (PC) is a nonfouling zwitterionic material that is based on the composition of the natural phospholipid components of the cell membrane [10,11]. It has been shown that surfaces coated with PC perform well as nonfouling coatings on multiple platforms [12–14]. Other zwitterionic functional groups have also been shown to have nonfouling properties. For example, Ladd et al. compared the nonspecific protein adsorption from 100% human serum and plasma on carboxybetaine (CB) and sulfobetaine (SB) surfaces to PEG based surfaces. Both zwitterionic surfaces had protein resistance on par with that shown by PEG upon exposure to these complex media [9]. The nonfouling properties of CB and SB based materials have been further demonstrated by others as well [15–19]. Molecular dynamics simulations have suggested that PC based materials form a strong hydration layer similar to PEG, this time through ionic solvation interactions [20]. It is assumed that this mechanism pertains to other zwitterionic functional groups.

Based on these results, it was suggested that any mixed charge system containing a homogeneous molecular level mixture of positively and negatively charged regions would also demonstrate nonfouling characteristics because of their similarity to zwitterionic materials. This was demonstrated with self-assembled monolayer investigations by others [21,22] and for polymeric based systems in our previous investigations [23,24]. In the first of these investigations a mixture of positively charged

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[2-(methacryloyloxy) ethyl] trimethyl ammonium chloride (TM) and negatively charged 3-sulfopropyl methacrylate potassium salt (SA) was found to be nonfouling only when the monomers were present in a 1:1 ratio [23]. Any slight deviation from the 1:1 ratio led to nonspecific adsorption from proteins with an opposite charge to the enriched monomer. Then it was demonstrated that mixed charge copolymers consisting of a positively charged [2-(acryloyloxy) ethyl] trimethyl ammonium chloride (TMA) and negatively charged 2-carboxy ethyl acrylate (CAA) monomers were also nonfouling. Furthermore, the TMA:CAA copolymers were shown to have a pH responsive bacteria "catch and release" property based on the protonation and deprotonation state of the carboxylic acid groups in the CAA monomer [24]. It is hypothesized that these responsive properties can be used to covalently attach proteins to TMA:CAA copolymer brushes using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide chemistry (EDC/NHS) while maintaining a nonfouling background away from the attached protein.

In this work, the interfacial properties of TMA:CAA copolymer brushes were further probed to gain insight into their multifunctional properties. The structure of these two monomers and the expected structure for the copolymer are shown in Scheme 1. The conditions used to complete surface-initiated atom transfer radical polymerization (ATRP) reactions were controlled to form TMA:CAA copolymers with varying thicknesses. Then the nonspecific protein adsorption from negatively charged fibrinogen (FBG) and positively charged lysozyme (LYZ) was probed to these copolymers using a surface plasmon resonance (SPR) biosensor. At the optimal thickness for nonfouling, nonspecific adsorption from 10% and 100% fetal bovine serum (FBS) was then quantified, to show the performance of these materials in complex media. Finally, the conjugation capacity of TMA:CAA copolymer brushes was demonstrated as a function of the brush thickness. The results obtained in this investigation clearly shown the dual functional capacity of TMA:CAA copolymer brushes, indicating their usefulness for biomaterial and biosensor applications.

2. Materials and methods

2.1. Materials

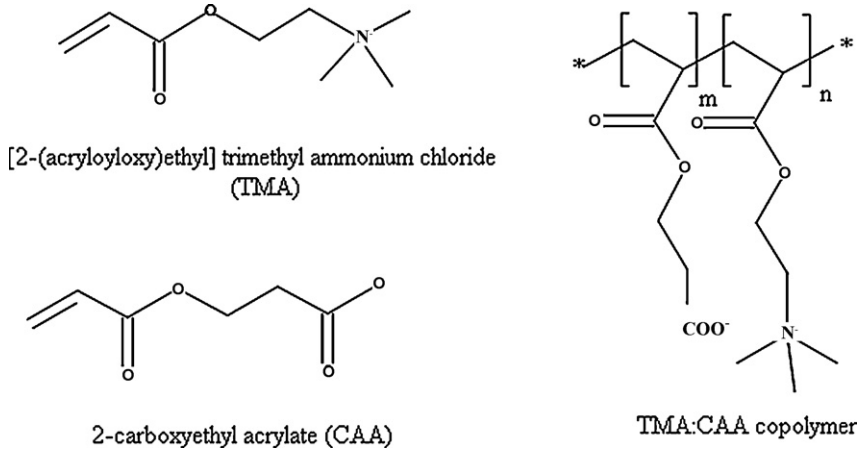
Bromoisobutyrate undecyl disulfide was purchased from Asemblon (Redmond, WA). [2-(acryloyloxy) ethyl] trimethyl ammonium chloride (TMA, 80 wt% solution in water), 2-carboxyethyl acrylate (CAA), copper (I) bromide (CuBrI), 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA), 150 mM phosphate buffered saline (PBS, pH 7.4), lysozyme (LYZ, >90%) from chicken egg white, N-hydroxysuccinimide (NHS, 98%), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, 99%), and tetrahydrofuran (THF) were purchased from Sigma–Aldrich (St. Louis, MO). Methanol (99%), NaOH, and copper (II) bromide (CuBrII) were purchased from Acros Organics (Geel, Belgium). Acetone and NaCl were purchased from Fisher Scientific (Pittsburg, PA). Ethanol (200 proof) was purchased from Decon Labs, Inc. (King of Prussia, PA). Borate buffer (20×) was purchased from Thermo Scientific (Waltham, MA). Fibrinogen (FBG, >95%) from bovine plasma was purchased from Calbiochem (Darmstadt, Germany). Fetal bovine serum (FBS) was obtained from Altanta Biologicals (Lawrenceville, GA). NaCl–PBS (pH 8.9) was prepared by adding sodium chloride to PBS (pH 7.4) to obtain a total concentration of NaCl of 0.5 M, followed by a pH adjustment to pH 8.9 with 1 M NaOH. The water that was used in all of the experiments was purified with a Millipore Synergy water system (Billerica, MA) to ultrapure conditions (18.2 MΩ-cm). Silicon wafers were purchased from University Wafer (South Boston, MA) and glass microscope slides were purchased from VWR International (Radnor, PA).

2.2. Self assembled monolayer formation

Substrates were coated with a 2 nm adhesion promoting titanium layer followed by a 48 nm gold layer using a sputter coater (AJA International, N. Scituate, MA). The gold coated chips were washed with ethanol and ultrapure water and then dried with filtered, compressed air. Then the chips were cleaned in a UV ozone cleaner (Jelight Inc., Irvine, CA) for 20 min. This was followed by another wash with water and ethanol. The chips were then immersed in a 0.1 mM bromoisobutyrate undecyl disulfide solution in pure ethanol. Following an overnight soak, the gold coated substrates were washed with THF, ethanol, and THF again and then they were dried with filtered, compressed air before placement in a reaction vessel.

2.3. Atom transfer radical polymerization (ATRP) reaction

Ultrapure water (5 mL) was added to a mixture containing 7 mmol of TMA and 7 mmol of CAA and then the pH of the solution was adjusted to pH 6.0 using 3 M NaOH. The TMA:CAA solution was deoxygenated for 20 min and then purged with nitrogen for an additional 20 min. HMTETA (400 μL) was degassed separately using three cycles consisting of 1 min of vacuum followed by 1 min of nitrogen purge. Then 10 mL of deoxygenated methanol was added to the HMTETA and 5 mL was added to the TMA:CAA solution. The



Scheme 1. Chemical Structures of the TMA and CAA monomers, and the TMA:CAA copolymer brush.

solutions were individually purged with nitrogen for an additional 20 min. The concentration of CuBrI was fixed at 1 mmol in all of the experiments, while the amount of CuBrII was varied to get the individual experimental CuBrII/CuBrI ratios. The copper components were added to the reaction vessel with the initiator coated gold substrates and then the vessel was deoxygenated. The HMTETA and TMA:CAA solutions were then moved to the reaction vessel using a syringe under nitrogen protection. The combined solution was purged with nitrogen for 20 min and then moved to a shaking water bath at a temperature of 25 °C. The reaction was allowed to proceed for 24 h or more depending on the particular reaction under investigation. Because similar reaction conditions have already been shown to produce TMA:CAA copolymers with a 1:1 surface composition, a detailed chemical composition analysis was not conducted [24].

2.4. Film thickness measurement

Silicon wafers were patterned with standard photolithography techniques in order to obtain 300 μm thick Au lines with a spacing of 300 μm. Briefly, a ~20 μm layer of primer and a 100 μm layer of Shipley 1813 photoresist (Rohm and Haas Electronic Materials, LLC, Philadelphia, PA) were spin-coated onto the wafers and they were soft baked for 1 min. The wafers were then placed under a photolithography mask that was patterned with 300 μm lines spaced 300 μm apart. After exposure to ultraviolet light, the sample was hard baked for 1 min and developed using a MF 319 developer (Rohm and Haas Electronic Materials, LLC). The samples were then coated with a 2 nm Ti adhesion layer and a 48 nm gold layer using a sputter coater. Finally, the samples were sonicated in acetone to remove the developed photoresist, leaving the 50 nm high, 300 μm wide patterned Au lines.

ATRP was conducted on patterned silicon wafer samples using the procedures outlined above. Then the dry thickness of the polymer brush was measured using a profilometer (Tencor Instruments, Milpitas, CA) by measuring the step height change of the pattern before and after polymerization. The thickness of the Au step was confirmed to be 50 nm. Following polymerization, anything above 50 nm was considered to be polymer. Just prior to taking the measurements, the surface of the sample was washed thoroughly with ultrapure water and dried with filtered, compressed air. A total of 3 measurements were taken from random locations on each of 2 independent samples ($n = 6$).

2.5. Protein adsorption using a surface plasmon resonance sensor

A custom built four-channel SPR biosensor based on wavelength interrogation was used to measure the amount of nonspecific protein adsorption (Institute of Photonics and Electronics, Prague, Czech Republic) [25,26]. The TMA:CAA coated SPR substrate was mounted to the SPR prism using a refractive index matching oil (Cargille Labs, Cedar Grove, NJ). Then a baseline was established using PBS buffer and 1 mg/mL solutions of either FBG or LYZ in PBS were injected into individual flow cells. The proteins were run for 10 min, after which the PBS buffer baseline was reestablished for 10 min. All of the solutions were run at a flowrate of 50 μL/min. The shift in the SPR signal from the initial to the final PBS buffer is directly proportional to the amount of nonspecific protein adsorption. For the SPR used in this investigation, a 1 nm shift at 750 nm is approximately equal to 17 ng/cm^2 of adsorbed protein [25]. To account for differences in the distance of the binding event from the surface plasmon active gold layer due to the varying brush thicknesses, the individual experimental results were scaled using a sensitivity factor. This factor was calculated for each experiment based on the starting wavelength for the initial PBS buffer baseline and a mathematical fit to a series of solutions to the Fresnel equations as solved for this sensor [26,27]. This approach more accurately accounts for subtle variations in the polymer brush thicknesses between individual experiments as compared to using an average value for a given thickness. The average sensitivity factors ranged from 1.01 to 1.18 (adjusted to an operating wavelength of 750 nm), which are similar to those used in studies with other polymer brush coatings [28–30]. The procedures for measuring the amount of nonspecific protein adsorption from both 10% and 100% FBS were identical. The 10% FBS was made by diluting the sample in PBS buffer. A total of 6 independent samples were analyzed for both FBG and LYZ at each TMA:CAA brush thickness and 3 independent samples were analyzed for both FBS conditions.

2.6. Fibrinogen conjugation using a surface plasmon resonance sensor

The conjugation of FBG was also monitored directly on the SPR sensor. Following the establishment of a PBS buffer baseline, the surface was activated for 7 min with a 2 mM NHS and 8 mM EDC solution. Following activation, a 1 mg/mL solution of FBG in borate buffer was run across the surface for 14 min. Then the surface was deactivated for 21 min with NaCl–PBS (pH 8.9). Finally, the PBS buffer baseline was reestablished. As before, the difference in the initial and final PBS buffer wavelengths is directly proportional to the amount of conjugated FBG. A total of 5 independent samples were analyzed for each TMA:CAA brush thickness.

3. Results and discussion

3.1. Varied polymer brush thicknesses

In order to probe the interfacial properties of TMA:CAA copolymers, it is essential to determine ATRP reaction conditions that lead to different polymer brush thicknesses. It is widely understood that by manipulating the ratio of CuBrII (slows the reaction down) to CuBrI (speeds the reaction up) it is possible to obtain surface initiated ATRP polymer brushes of differing thicknesses [28,31,32]. In this investigation the CuBrII/CuBrI molar ratio was varied from 20% to 0.5% with all other reaction conditions held constant. As shown in Fig. 1a, it can be seen that the thickness of the copolymer gradually increases from 5 nm to 18 nm as the ratio is decreased from 20% to 0.5%.

While a thicker polymer brush may have been obtained by eliminating CuBrII, it is ideal to have trace amounts of this component to help the reaction to occur more uniformly across the surface [32]. Instead, to obtain polymer brushes with thicknesses that were even greater than 18 nm, the reaction time was increased for the 0.5% CuBrII/CuBrI ratio. The influence of reaction time on the polymer brush thickness can be seen in Fig. 1b. The maximum thickness that was obtained in this study was 50 nm for a 0.5% CuBrII/CuBrI ratio over a 72 h reaction period. The results from the profilometry experiments also qualitatively confirmed that a uniform surface coverage was obtained for all of the different ATRP reaction conditions examined here (data not shown).

3.2. Simple protein solution nonfouling studies

It has been previously demonstrated that the nonfouling properties of polymer brushes are directly dependent upon the polymer brush thickness, with a composition specific optimal thickness for nonfouling [28–30]. This thickness dependency has been attributed to a conformational phase transition from a non-associated chain state to either an inter- or intra-chain association state that leads to the collapse of the polymer brush [33]. When nonfouling polymer brushes are too thin, it has been hypothesized that the proximity

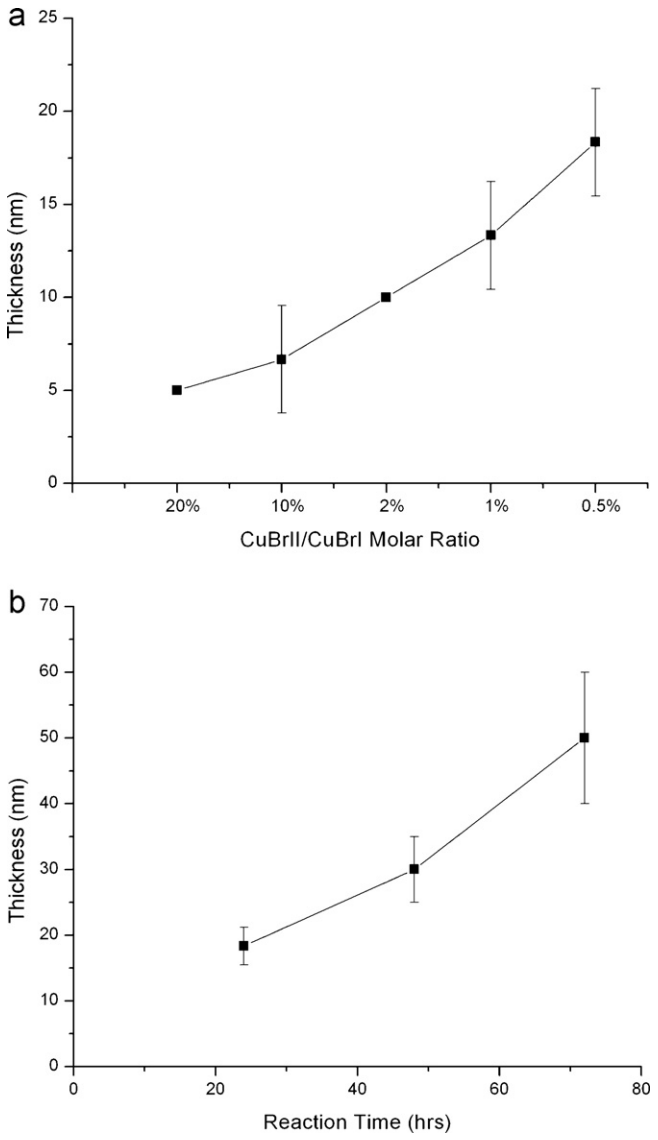


Fig. 1. Average ± standard deviation of measured TMA:CAA brush thicknesses under varying reaction conditions ($n = 6$). (a) TMA:CAA brush thickness as a function of the ratio of CuBrII/CuBrI following a 24 h ATRP reaction; and (b) TMA:CAA brush thickness as a function of reaction time with a CuBrII/CuBrI ratio of 0.5%. The data point representing the 0.5% CuBrII/CuBrI and 24 h reaction condition is repeated in both figures.

of the functional groups is not close enough to support the formation of a strong hydration layer. As the thickness increases, these functional groups becomes more dense and uniform, promoting the formation of a strong hydration layer that is believed to mediate the nonfouling properties. Finally, when the brush transitions to its inter- or intra-chain association state, the functional groups interact with each other more strongly, leading to a disruption of the hydration layer and a subsequent increase in the nonspecific protein adsorption [30]. In a study by Azzaroni et al., contact angle measurements on SB coated surfaces showed that the surface underwent a transition from a hydrophilic state to a hydrophobic state as a function of polymer brush thickness [33]. In an unrelated study into the thickness dependency of the nonfouling properties by Yang et al., the optimal thickness for nonfouling was seen to fall approximately in the middle of the hydrophilic/hydrophobic transition range identified by Azzaroni [28,33]. These studies support the hypothesis that the optimal thickness for nonfouling is strongly related to the ability of the underlying polymer brush coating to tightly bind its hydration layer.

The hypothesis that the ability of a surface to tightly bind water dictates its nonfouling properties is also consistent with results shown by multiple investigators into the role that polymer brush density has on the nonfouling properties. For example, Feng et al. demonstrated that there was a significant difference in the advancing contact angle found for a given PC chain length and different graft densities. These results correlated with nonspecific protein adsorption studies that confirmed that for a given chain length, there was an optimal grafting density that minimized the nonspecific protein adsorption [13]. These results are also consistent with those demonstrated by others for polymer brush coatings [34–36] and self-assembled monolayer coatings [37,38].

The nonspecific adsorption of FBG and LYZ was tracked with an SPR biosensor to determine the optimal thickness for nonfouling for the TMA:CAA copolymer brush. These two proteins were selected as model proteins because of their wide use in novel nonfouling material screening and because they are oppositely charged at neutral pH [23,28,39]. The results in Fig. 2a and b show that the nonspecific adsorption of LYZ and FBG follow the expected trend of higher protein adsorption at low polymer brush thicknesses, dropping through a minimum point, and then slowly increasing again. In

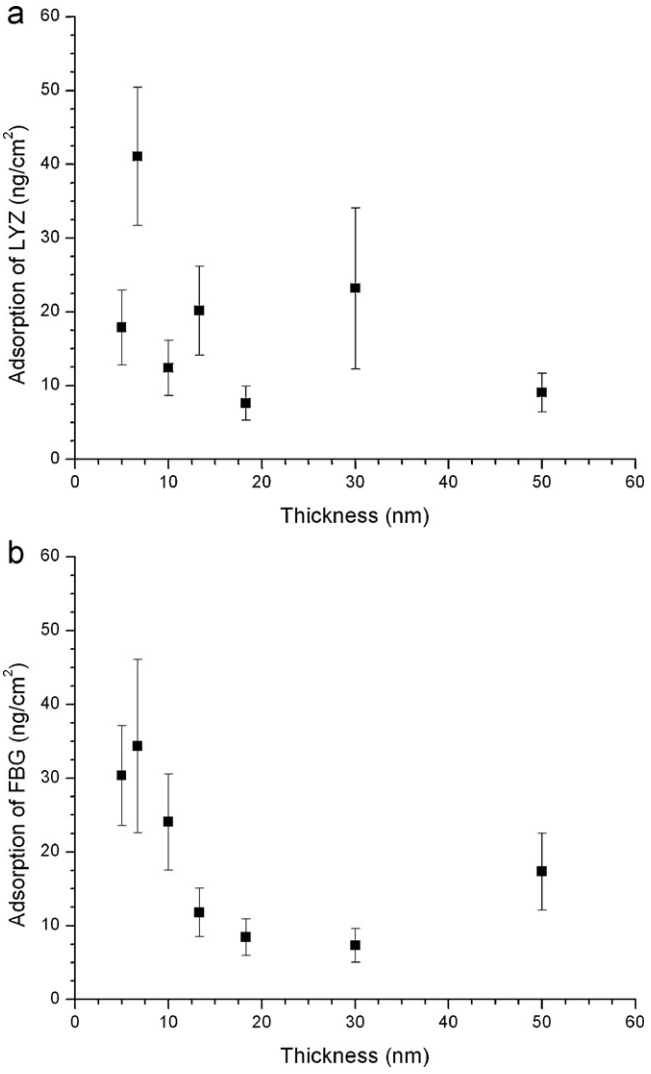


Fig. 2. Average ± standard error of the mean of nonspecific adsorption of: (a) LYZ and (b) FBG to TMA:CAA brush coatings as measured by SPR. Six independent experiments were run at each copolymer brush thickness ($n = 6$).

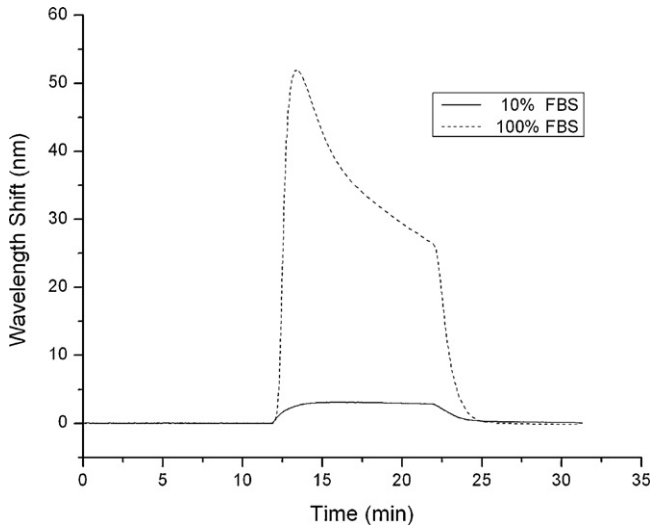


Fig. 3. Representative SPR sensorgrams showing the nonspecific adsorption from 10% and 100% FBS to a TMA:CAA copolymer brush at the optimal nonfouling thickness of 18 nm.

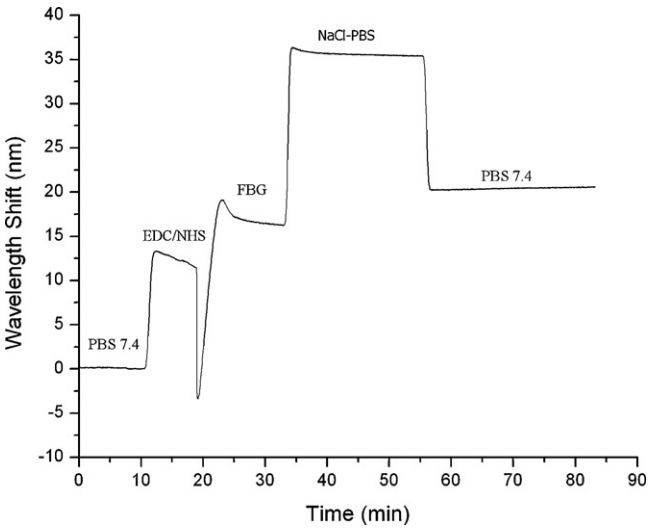


Fig. 4. Representative SPR sensorgram showing the sensor response during the activation, conjugation of FBG, and deactivation of a TMA:CAA copolymer brush.

Fig. 2a, the ideal TMA:CAA copolymer brush thickness for preventing the nonspecific adsorption of LYZ is seen to be ~18 nm. In Fig. 2b, the best point is seen to fall somewhere in the range of 18–30 nm. Because of the increased levels of LYZ adsorption found on brushes of 30 nm, it was concluded that the ideal TMA:CAA copolymer brush thickness for maximizing the nonfouling properties falls closest to ~18 nm, which was obtained with a 0.5% CuBrII/CuBrI ratio over a 24 h reaction period.

3.3. Fetal bovine serum nonfouling studies

Preventing nonspecific protein adsorption from a complex media is much more challenging than from single protein solutions, due to the wide range of proteins and other biological molecules present. In order to compare the nonfouling performance of TMA:CAA copolymer brushes at the optimal thickness of ~18 nm to other nonfouing functional groups, FBS was selected as a test medium. Nonspecific protein adsorption from both 10% and 100% FBS was tracked using an SPR biosensor and Fig. 3 shows representative SPR sensorgrams for those tests. In this figure it can be seen that there is a large initial response upon injection of 100% FBS that slowly decreases with time. This response can likely be attributed to on-going reversible interactions between components of the FBS and the TMA:CAA copolymer brush. However, upon reinjection of the PBS buffer it can be seen that the baseline is quickly reestablished with minimal net irreversible binding. The average (±standard deviation) nonspecific adsorption was determined to be $2.1 \pm 2.0\,ng/cm^2$ and $4.3 \pm 1.7\,ng/cm^2$ from 10% and 100% FBS, respectively ($n = 3$). These results indicate that TMA:CAA copolymer brushes have ultralow fouling properties that are on par with other more established nonfouling functional groups. For example, Zhao et al. demonstrated that poly(hydroxy ethyl methacrylate (polyHEMA) had nonspecific protein adsorption on the order of $3.0\,ng/cm^2$ from human blood serum when at the optimal thickness for nonfouling [30]. Additionally PEG coated surfaces have been shown to have nonspecific protein adsorption from FBS below the limit of detection (<1 Å) when measured by elipsometry [40]. Feng et al. have also demonstrated that PC based coatings have nonspecific adsorption of FBG around $5\,ng/cm^2$ at the optimal chain grafting density and length [13]. It is also interesting to note that the optimal thickness for nonfouling found here was within one standard deviation of that used in our previous study that showed the strong resistance of TMA:CAA copolymer brushes to bacteria adhesion [24]. This further supports the conclusion that TMA:CAA copolymer brushes of ~18 nm have excellent nonfouling properties to both simple and complex media.

3.4. Covalent protein attachment studies

The TMA:CAA copolymer presents a unique advantage over many other nonfouling functional groups, with the exception of CB based materials. CB based materials have been widely demonstrated to have a dual functional property that allows for the covalent attachment of proteins within a nonfouling background using EDC/NHS conjugation chemistry [19,41,42]. No other nonfouling functional groups have been demonstrated to have this functionality without compromising their native nonfouling properties. It was hypothesized that TMA:CAA copolymers would have a similar property based on the presence of carboxylic acid groups in the CAA monomer side chain and the fact that TMA:CAA copolymers have a pH dependent response that was stronger than that of CB based polymers [24]. The ability of TMA:CAA copolymers to

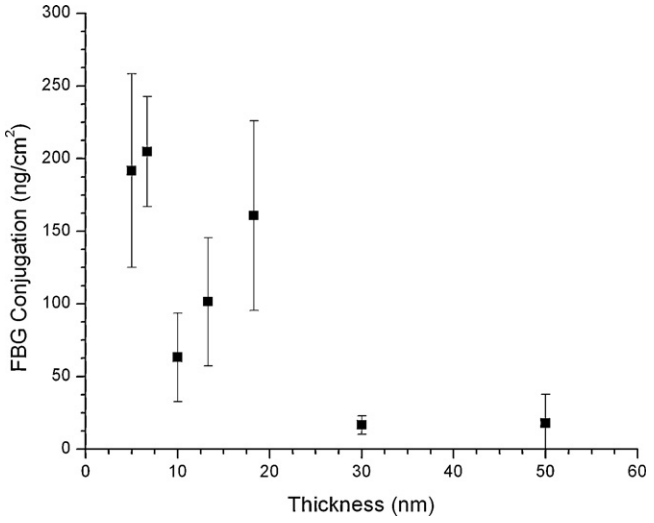
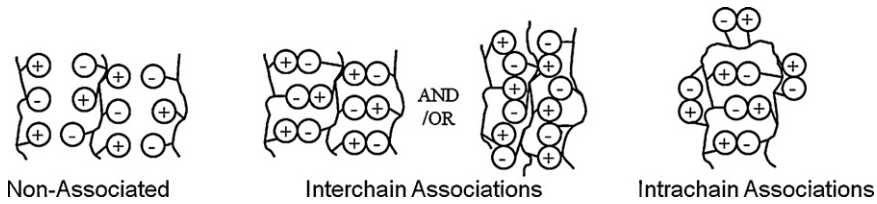


Fig. 5. Average ± standard error of the mean of FBG conjugation to TMA:CAA copolymer brushes as measured by SPR. Five independent experiments were run at each copolymer brush thickness ($n = 5$).



Scheme 2. Potential conformational states of the TMA:CAA copolymer brush coatings that influence the nonfouling and protein conjugation properties.

covalently attach proteins using EDC/NHS chemistry was investigated here as a function of the polymer brush thickness using an SPR biosensor. FBG was selected as a model protein for investigating the conjugation capabilities because of its role in mediating cellular adhesion to biomaterial surfaces [43] and because it was used as one of the probe proteins in the nonfouling portion of this study. This allows for further insight to be gained about the role of the polymer brush because the results can be compared directly to the nonfouling results presented in Fig. 2b. In order to conjugate proteins, the surface must first be exposed to an acidic solution to protonate the carboxylic acid groups, to allow for NHS activation. Following conjugation, unreacted carboxylic acid groups must be deprotonated with a basic buffer rinse, to return the surface to its native nonfouling state. The SPR response to each of the activation, conjugation, and deactivation steps can be seen in the representative SPR sensorgram shown in Fig. 4.

The results in Fig. 5 show the levels of FBG conjugation that were obtained at the various TMA:CAA copolymer brush thicknesses. The results show an interesting pattern where the conjugation level initially starts out high, drops to a local minimum at thicknesses of 10 nm (68.0 ng/cm^2 of conjugated FBG) and increases through the ~18 nm thick brush. At the optimal thickness for nonfouling, the TMA:CAA copolymer brush was found to be capable of conjugating 160.7 ng/cm^2 of FBG. As the brush thickness is further increased, the conjugated protein level again drops to a minimum. These results indicate that the TMA:CAA copolymer brush coating represents a promising biomaterial platform for combining protein conjugation capacities with a nonfouling background as long as the polymer brush is shorter than the critical phase transition thickness. It should also be mentioned that this level of protein conjugation was achieved with limited efforts to optimize the conjugation chemistry and it may be possible to improve it further [41].

The initial high levels of FBG conjugation on the shortest TMA:CAA brushes may actually represent the combination of conjugated protein plus nonspecific FBG adsorption to the background. At the lowest thicknesses, the TMA:CAA copolymer did have significant fouling as shown in Fig. 2b and this is still present here. The increase in the amount of conjugated FBG on the TMA:CAA copolymer brushes with thicknesses ranging from 10 nm to 18 nm is likely due to the increasing number of carboxylic acid groups present and available for conjugation, while the copolymer brush chains are still in a non-associated conformation. This keeps the carboxylic acid groups accessible and available for activation and conjugation. The strong drop off in the levels of conjugated protein to the thickest two TMA:CAA brushes is likely due to a conformation change from the non-associated state to an intra- or inter-chain association state [33,44]. As the quaternary amine and carboxylic acid groups interact more strongly, it will be increasingly unfavorable for the carboxylic acid groups to undergo activation and conjugation. This is shown conceptually in Scheme 2, which shows the non-associated and associated conformations of the copolymer that are possible. On-going efforts are focused on characterizing the functional group interactions on the surface as a function of the TMA:CAA copolymer thickness to test this hypothesis.

4. Conclusions

In this investigation, the interfacial properties of TMA:CAA copolymer brushes formed by surface-initiated ATRP were investigated. The optimal thickness that minimized nonspecific protein adsorption from simple FBG and LYZ solutions was determined to be 18 nm. At this thickness, the TMA:CAA copolymer brush was shown to have ultralow fouling properties even upon exposure to 100% FBS (<5 ng/cm^2 of nonspecific protein adsorption). The dual functional properties of TMA:CAA copolymers were also demonstrated with protein conjugation studies. It was shown that the conjugation capacity of the TMA:CAA copolymer is dependent upon the brush thickness and is eliminated when the brush collapses upon itself at the onset of the intra- and inter-chain association conformation. However, at the optimal thickness for nonfouling, the brush was capable of conjugating ~150 ng/cm^2 of FBG, indicating its potential for use as a multi-functional biomaterial platform.

Acknowledgements

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