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Carboxybetaine functionalized nanosilicas as protein resistant surface coatings

Abstract

Materials with protein resistant properties are increasingly sought after for their potential application as low-fouling surface coatings. Hydrophilic coatings with improved resistance to protein fouling have been prepared from zwitterionic carboxybetaine (CB) functionalized silica nanoparticles (SiNPs). The authors report three methods of coating preparation via direct tethering of CB to predeposited particle films, a two-step surface functionalization process, and deposition of CB functionalized particle dispersions. The pH at which aqueous CB solutions were prepared and reacted to SiNPs was found to drastically influence the mechanism of CB attachment and affect the protein resistance of the resultant coatings. Depending on the method of coating preparation, protein binding to functionalized particle coatings was reduced by up to 94% compared to unfunctionalized SiNP control surfaces. As a result, all three methods offer simple and scalable fabrication routes for the generation of hydrophilic, zwitterionic interfaces with improved inhibition to protein fouling.

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ABSTRACT

Materials with protein resistant properties are increasingly sought after for their potential application as low-fouling surface coatings. Hydrophilic coatings with improved resistance to protein fouling have been prepared from zwitterionic carboxybetaine (CB) functionalized silica nanoparticles (SiNPs). The authors report three methods of coating preparation via direct tethering of CB to predeposited particle films, a two-step surface functionalization process, and deposition of CB functionalized particle dispersions. The pH at which aqueous CB solutions were prepared and reacted to SiNPs was found to drastically influence the mechanism of CB attachment and affect the protein resistance of the resultant coatings. Depending on the method of coating preparation, protein binding to functionalized particle coatings was reduced by up to 94% compared to unfunctionalized SiNP control surfaces. As a result, all three methods offer simple and scalable fabrication routes for the generation of hydrophilic, zwitterionic interfaces with improved inhibition to protein fouling.

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I. INTRODUCTION

The antifouling properties of zwitterionic polymers have been largely attributed to their exceptional hydration capacity. Zwitterions bind water about their charged groups via electrostatic interactions, generating an interfacial hydration layer that can prevent interactions and adsorption of biomolecules and microbial organisms.^{1–3} Studies investigating zwitterion hydration by molecular modeling have demonstrated that the residence time, orientation, and number of water molecules associated with zwitterionic chemistries is dependent upon the identity and electrochemical properties of the charged groups present.^{4–6} Theoretical simulations comparing zwitterionic carboxybetaine (CB) and sulfobetaine (SB) have shown that the sulfonate group (SO_3^-) of SB coordinates a larger number of water molecules than the carboxylate group (CO_2^-) of CB, while CB coordinated water molecules have sharper spatial distribution and longer residence times.⁶ It is anticipated that these subtle differences in hydration capacity would influence

the ability of these materials to prevent biological fouling. Indeed, previous studies have demonstrated that SB modified cellulose membranes have better protein resistance than CB modified surfaces,⁷ while polymeric CB modified surfaces have shown improved resistance to nonspecific protein adsorption from human blood plasma and serum over polymeric SB and self-assembled mixed charge zwitterionic surfaces.⁸

In addition to differences in hydration capacity, the electro-negativity of zwitterionic anionic groups will influence their response to media and variation in pH. The sulfonate group of SB is a weak base and will remain negatively charged even at low pH; however, the carboxylate group of CB is a strong conjugate base (the carboxyl group is a weak acid with a typical pK_a between 3 and 5) and is susceptible to protonation under low pH conditions.⁹ As a result, the zwitterionic character and resultant antifouling properties of CB modified surfaces will be dependent upon the pH conditions of preparation and exposure.

A number of different surfaces, including glass,^{10–12} gold,^{13–15} PVDF,^{16,17} and cellulose membranes,^{7,18,19} have been functionalized with zwitterionic polymers to improve fouling resistance. Where surfaces do not directly present reactive surface groups for functionalization, alternate methods are sought to introduce zwitterionic chemistries to the interface. We have previously reported on the preparation of hydrophilic, low-fouling coatings from SB functionalized silica nanoparticles (SiNPs), where SiNPs could be functionalized either before or after their deposition onto surfaces for improved fouling resistance.²⁰ In the present study, three methods were employed to generate SiNP coatings presenting zwitterionic CB functionality. As the protein resistant properties of CB are well documented, this study focused on validating the preparation of CB functionalized SiNP coatings by simple and scalable methods. The results presented herein highlight the versatility of SiNP coating systems, demonstrating that coatings can be prepared utilizing different methods depending on the desired application.

Silanated CB was used to directly modify the surface of SiNP films, where conditions of reaction (i.e., pH and solution concentration) were monitored using a quartz crystal microbalance with dissipation monitoring (QCM-D). SiNP coatings were also modified utilizing a two-step process, eliminating the need for preparation of the CB monomer species separately. Additionally, suspensions of SiNPs were functionalized and deposited as thin films for the investigation of their antifouling properties. All of the prepared surfaces were hydrophilic and showed improved resistance to protein binding. We demonstrate that the self-assembly or polymeric type organization of CB chemistries onto the SiNP surfaces is pH dependent and influences the mechanism of protein resistance.

II. EXPERIMENT

A. Materials

Ludox® HS-40 colloidal silica (12 nm diameter), poly(ethyleneimine) solution (PEI, P3143), (*N,N*-dimethylaminopropyl)trimethoxysilane, acetone (270725), bovine serum albumin (BSA) (A-3059), and phosphate buffered saline (PBS) were purchased from Sigma-Aldrich. Acetone was dried with 3 Å molecular sieves and distilled before use. β -propiolactone (PL) (H0168) was purchased from Tokyo Chemical Industry. All other reagents purchased from commercial suppliers were used without further purification. Water used to prepare aqueous solutions was purified using a Millipore water purification system with a minimum resistivity of 18.2 M Ω cm at 25 °C.

B. Synthesis of zwitterionic CB

The synthesis of silanated CB [3-(dimethyl(3-(trimethoxysilyl)propyl)ammonio)propanoate] was adapted from Hu *et al.*²¹ (*N,N*-dimethylaminopropyl)trimethoxysilane (2.08 g, 0.01 mol) was added dropwise to β -propiolactone (0.72 g, 0.01 mol) in anhydrous acetone (5 ml) under Ar. The reaction was stirred under Ar for 24 h and resulted in the formation of a white precipitate. The unreacted material was removed by a rotary evaporator. The resulting material was collected and dried at 70 °C under vacuum. ¹H NMR (400 MHz, D₂O): δ 3.53 (t, 2H), 3.31 (s, 9H), 3.06 (s, 6H),

2.85 (m, 2H), 2.63 (m, 2H), 1.86 (quin, 2H), 0.69 (t, 2H). IR (cm⁻¹): 1728, 1582, 1485, 1373, 1195, 1061, 930.

C. Preparation of SiNP coatings

Silica nanoparticle solutions were prepared as 4 wt. % dispersions in water for spin-coating onto gold QCM sensors. Two types of coatings were prepared. First, dispersions of unreacted SiNPs were prepared for fabrication of nanoparticle coatings that would undergo functionalization within the QCM. Second, coatings were prepared from dispersions of CB functionalized SiNPs for assessment of their antifouling properties. Coatings of the SiNP dispersions were prepared onto A-T cut quartz crystal microbalance sensors with a 10 mm diameter gold electrode (QCM-D, Q-Sense AB Västra, Frölunda, Sweden) and a fundamental resonance frequency of 5 MHz. Prior to coating, the gold sensor surface of each sensor was cleaned with piranha solution [7:3 v/v mixture of H₂SO₄ (98%) and H₂O₂ (33%)] for 3 min, rinsed with de-ionized water, and dried in a stream of nitrogen gas. Cleaned QCM-D sensors were then incubated in 0.5% PEI solution for 10 min to form an adhesive layer on the electrode surface. The PEI was thoroughly rinsed from the sensors with de-ionized water, and the sensors were dried in a stream of nitrogen gas. The QCM sensors were mounted onto glass slides, and nanoparticle films were prepared by depositing 20 μ l of each of the prepared nanoparticle dispersions onto the gold electrode of the sensor and spin-coating at 5000 rpm for 30 s. Coatings were cured for 1 h at 120 °C and thereafter thoroughly rinsed with de-ionized water and dried in a stream of nitrogen gas. SiNP coatings possessed good durability as demonstrated by acoustic emission critical load measurements (10 N/m) of coating failure, when deposited on commercial polymer coating systems (data not shown). The cross-sectional thickness of SiNP coatings prepared in this manner was $\sim$ 100 nm.

D. Quartz crystal microbalance (QCM-D)

CB adsorption onto SiNP coated gold sensors and protein adsorption experiments onto the CB functionalized SiNP coatings were carried out by a Q-Sense E4 Quartz crystal microbalance with dissipation monitoring (QCM-D, Q-Sense AB Västra, Frölunda, Sweden) coupled with an ISMATEC IPC High Precision Multichannel Dispenser (IDEX, Wertheim, Germany) in a flow-through setup. Q-SENSE QTOOLS analysis software v3.0.10.286 (Biolin Sci, AB) was used to apply the Voigt model to calculate the mass of CB or adsorbed protein onto the modified sensor surface. The following input parameters provided the best fit for the layer density (1150 kg/m³), fluid density (1020 kg/m³), layer viscosity ($10^{-6} \leq 10^{-2}$ kg/m s), layer shear modulus ($10^4 \leq 10^8$ Pa), and mass ($1.15 \leq 1.155$ ng/cm²). The third, fifth, and seventh overtone *f*/*D* responses were used for modeling calculations.

E. Methods of preparing CB functionalized SiNP coatings

1. CB functionalization of SiNP coatings by QCM-D

Adsorption of CB onto SiNP coated gold QCM sensors was monitored by the QCM-D. SiNP coated sensors were used without any further surface treatment. Aqueous solutions of CB monomer

(1.0 and 10 mM) were prepared immediately prior to experiments and pH adjusted as necessary. Solutions were introduced into the QCM chamber at a flow rate of 60 μ l/min, and surfaces were exposed for a period of 30 min. Sensors were then rinsed with de-ionized water at the same flow rate for a further 30 min or until the QCM measurement parameters stabilized. All experiments were run in triplicate.

2. Two-step CB functionalization of SiNP coatings

The procedure for the two-step surface functionalization of SiNP coatings with CB was adapted from Huang and Chang.¹¹ SiNP coated sensors were first immersed in a 20 mM solution of (*N,N*-Dimethylaminopropyl)trimethoxysilane in EtOH with 2% addition of H₂O for 6 h. Substrates were removed from solution, rinsed with EtOH, and dried in a stream of nitrogen gas. Substrates were then cured in an oven at 120 °C for 1 h. A 10 mM solution of β -propiolactone in acetonitrile was prepared, and the silane modified substrates were incubated in this solution at 4 °C for 6 h. Coatings were rinsed with acetonitrile and dried in a stream of nitrogen gas.

3. CB functionalization of SiNP dispersions

The appropriate amount of CB monomer for reaction with SiNPs was calculated based on the surface area reported by the manufacturer and 4.9 silanol groups per square nanometer of silica surface.²² CB was dissolved in H₂O, and the pH was adjusted to 10 by the addition of NaOH. The CB solution was added dropwise to a stirred solution of SiNP with a final particle concentration of 10 wt. %. The reaction mixture was stirred for 6 h. Thereafter, particle solutions were placed into 12 K molecular weight cutoff dialysis membrane and dialyzed against pure water for 5 days with water changed regularly to remove unreacted CB monomer. Particles were freeze-dried for further characterization.

F. Characterization

1. Fourier transform infrared (FTIR) spectroscopy

FTIR spectra were acquired on a Shimadzu Prestige-21 Fourier transform infrared spectrophotometer with a PIKE MIRacle ATR attachment.

2. Nuclear magnetic resonance (NMR) spectroscopy

¹H NMR (400 MHz) spectra were obtained on an AVANCE III HD NMR Spectrometer (Bruker) at 25 °C using D₂O as a solvent.

3. Dynamic light scattering (DLS)/zeta potential (ZP)

The Z-average particle size (hydrodynamic particle diameter) was measured by DLS, and ZP measurements were performed on a Malvern Zetasizer Nano-ZS at 25 °C (colloidal silica refractive index: 1.40, absorption: 0.010, measurement angle: 173°). ZP measurements were performed on 0.5 wt. % aqueous particle solutions, and the pH was adjusted with NaOH or HCl addition. DLS measurements were carried out on 0.5 wt. % particle solutions dispersed in 10 mM NaCl at pH 9.5.

4. Thermogravimetric analysis (TGA)

The quantity of zwitterion bound to the particle surface was determined by thermogravimetric analysis (TA, Q500). Dried nanoparticles were heated from room temperature to 800 °C at a heating rate of 10 °C/min under a nitrogen atmosphere. The mass of polymer bound to the particle surface was calculated from the weight loss measured between 150 and 800 °C. The mass retained at 800 °C after polymer decomposition was assumed to be the mass of bare SiNPs.

5. Contact angle goniometry

The mean static contact angle (CA) made by a 2 μ l sessile water droplet on the surface of each of the particle coatings was measured with a Dataphysics Contact Angle System (OCA 15EC) in conjunction with sCA20 software. A minimum of three measurements were obtained for triplicate samples of each particle coating.

6. Scanning electron microscopy (SEM)

Imaging of particle coatings was performed using field emission SEM (JEOL JSM-7500FA). Images were obtained without further modification of the particle coating surface.

G. Protein adsorption

SiNP coated sensors were placed into standard Q-Sense flow modules (QFM 401) and equilibrated in PBS for a minimum of 1 h at a constant temperature of 22.00 $\pm$ 0.02 °C. 1 mg/ml solution of BSA in PBS was introduced into the flow chamber at a constant flow rate of 60 μ l/min for 30 min. Sensors were subsequently rinsed with PBS for a further 30 min at the same flow rate or until QCM measurement parameters stabilized. All experiments were run in triplicate.

H. Statistical analysis

Numerical results are expressed as means $\pm$ 95% confidence intervals. Results were analyzed using one-way analysis of variance (ANOVA) with Tukey *post hoc* test. Probabilities of *p* < 0.05 were considered to be significantly different. All statistical analyses were performed using IBM SPSS STATISTICS 21 software.

III. RESULTS AND DISCUSSION

A. CB functionalization of SiNP coatings by QCM-D

The CB species used throughout this study was prepared via a nucleophilic ring-opening reaction of β -propiolactone by an aminosilane (AS), generating a quaternary ammonium cation and carboxylate anion (Fig. S1 in the supplementary material³³). The resulting product was very hygroscopic, consistent with previous reports in the literature.^{23,24} Thin films of SiNPs were cast onto gold QCM sensors via spin-coating, where they generated a uniform, hydrophilic, and reactive substrate for functionalization with CB (Fig. S2 in the supplementary material³³). Sensors were placed into QCM modules and equilibrated in de-ionized water until frequency (*f*) and dissipation (*D*) parameters stabilized. Solutions of CB at concentrations of 1 and 10 mM were prepared immediately prior to their introduction into the QCM and pH

adjusted to 3.5, 7.0, and 9.5 with the addition of dilute NaOH or HCl. Gradual decreases in f were observed on exposure to 1 mM CB solutions, with decreasing pH resulting in larger negative f shifts [Fig. 1(a)]. Increasing the solution concentration to 10 mM dramatically increased the rate of binding for CB solutions of pH 7.0 and 9.5, where large initial f shifts were observed before the rate of adsorption slowed and plateaued [Fig. 1(b)].

For acid-catalyzed functionalization of SiNP coatings at both 1 and 10 mM solution concentrations, the rate of CB attachment slowed but did not level off during the 30 min exposure period. Based on this observation, it was thought that the attachment mechanism occurring under low pH conditions may differ significantly from those occurring at neutral and alkaline pH. The magnitude of the f response to CB under acidic conditions could indicate that uncontrolled surface polymerization reactions are occurring in place of monolayer self-assembly, resulting in large adsorptions at the coating interface. The mass of covalently bound CB was calculated by fitting the Voigt-based viscoelastic model, accounting for dissipative energy losses due to water associated with highly hydrated polymers. As anticipated from the raw QCM frequency responses, the mass of adsorbed CB increased with decreasing pH and with increasing concentration [Fig. 1(c)]. For both 1 and 10 mM functionalization concentrations, the differences in adsorbed CB across the three pH conditions were all found to be significantly different (one-way ANOVA, $p < 0.05$).

Previous reports in the literature have shown that the hydrolysis of alkoxysilanes and the condensation reactions that bind them to surfaces can be facilitated under both high and low pH conditions.^{25–28} Most reports agree that hydrolysis and condensation reactions occur rapidly at basic pH; however, high pH conditions can also promote self-condensation in solution, limiting the number of molecules available for surface reaction or causing surface crowding through attachment of oligomers. In this way, the degree of surface functionalization could be reduced, as was observed for CB mass binding at pH 9.5. While the total mass of CB attachment was significantly less at pH 9.5 than at pH 7.0, the similar f attachment profiles observed in the QCM lead us to believe that CB self-assembly onto the SiNP surface was occurring via the mechanism proposed in Fig. 2(a).

Acidic conditions have been shown to promote hydrolysis but slow the rate of self-condensation reactions,²⁵ allowing for the organization of tightly packed and uniform self-assembled monolayers. However, the distinct f attachment profile and large mass adsorption of CB at pH 3.5 indicate that surface reactions under acidic conditions are not forming self-assembled monolayers but instead may be generating a polymeric type network at the sensor surface.

It is proposed that at low pH the carboxylate group of CB will become protonated and silane-ester condensation reactions can occur. Similar acid-catalyzed ester condensation reactions have been reported by Schmidt *et al.*, where carboxylic acid groups reacted with free silanol groups on a bare silicon oxide surface and unreacted silanol groups of other anchored organosilanes.²⁹ In this way, functionalization of the SiNP surface could occur either through standard silanol coupling reaction with the surface or via carboxylic acid reaction with silanol groups on the SiNP surface [Fig. 2(b)].

Assuming self-assembly of CB proceeds in this way under acidic conditions, it is anticipated that organization of an initial

FIG. 1. Raw QCM f shifts (fifth overtone) of SiNP coatings on exposure to 1 mM (a) and 10 mM (b) CB solutions adjusted to pH 3.5, 7.0, and 9.5. Mass of CB adsorbed onto SiNP coatings with varied concentration and pH (c). Error bars represent 95% confidence intervals about the mean. Asterisks (*) indicate statistically similar subsets.

FIG. 2. Proposed reaction scheme of CB attachment occurring via standard silanol coupling reactions under neutral and basic conditions (a) and CB attachment occurring via reaction of protonated carboxylate groups with free silanol groups on the SiNP surface (b).

monolayer at the SiNP surface would present a random assortment of oppositely orientated CB molecules. This would result in the presentation of two available sites for further reactions. As shown in Fig. 3, condensation reactions may propagate from either free silanols or protonated carboxyl groups, leading to uncontrolled polymerization type reactions and large mass adsorptions at the QCM sensor surface.

Water CAs of the QCM prepared SiNP coatings were measured after functionalization with CB. Typically, SiNP coatings are already very hydrophilic, presenting CA's of $\sim 10^\circ$. Reaction with 10 mM CB at pH 9.5 did not drastically affect the measured contact angle; however, slight increases were observed after reaction with CB at pH 3.5 and 7.0 (Fig. S3 in the supplementary material³³). Despite the increased mass binding at pH 3.5 and 7.0, it is thought that the basic nature of the carboxylate group could lead to protonation at these pH conditions, resulting in (partial) loss of zwitterionic character and a subsequent increase in the measured contact angles. This same trend was observed for reaction with CB at 1 mM concentration; however, the increase in CA was not as pronounced (Fig. S4 in the supplementary material³³).

FIG. 3. Proposed mechanism of uncontrolled polymerization type reactions propagating from oppositely orientated CB molecules under acidic conditions.

B. Two-step CB functionalization of SiNP coatings

An alternate method of generating zwitterionic CB interfaces in the form of a two-step process was also explored. By preparing surfaces in this way, the preparation and storage of the highly hygroscopic CB monomer can be avoided. In this method, SiNP films were first functionalized with the AS species and then exposed to PL to generate a zwitterionic interface (Fig. 4).

SiNP coatings were exposed to a solution containing 20 mM (*N,N*-dimethylaminopropyl)trimethoxysilane in EtOH with 2% addition of H₂O. Previous studies have demonstrated that hydrolysis of amino alkoxy silanes occurs rapidly in EtOH/H₂O mixtures and that increasing temperature enhances the rate of self-condensation reactions.^{26,28} For this reason, substrates were cured briefly after exposure to AS (120 °C, 1 h) to enhance stability through promotion of condensation reactions between bound

FIG. 4. Schematic of the two-step surface functionalization of SiNP coatings (top). Representative images of water contact angles made with the surface of SiNP coatings (bottom): before functionalization, after reaction with AS, and after further reaction with PL. Insets: Average contact angles measured on the prepared surfaces (± SD).

chemistries. After exposing SiNP surfaces to the AS moiety, the measured water contact angle increased from 9.5° to 47.6° , indicating successful surface functionalization (Fig. 4). AS-SiNP surfaces were then exposed to a 10 mM solution of PL in acetonitrile at 4°C , where a nucleophilic ring-opening reaction was anticipated to generate the desired zwitterionic interface. This reaction had to be carried out with the exclusion of water, as PL is highly susceptible to hydrolysis and will generate 3-hydroxypropionic acid. Additionally, PL can polymerize at room temperature, and thus the reaction was performed at reduced temperature. Contact angles were measured again after reaction with PL and were found to have decreased to 29.5° . This reduction in contact angle indicated that the surface had become more hydrophilic; however, the measured contact angle was higher than what might be anticipated for traditional zwitterionic materials. This may indicate that the second stage of reaction did not result in complete conversion to a zwitterionic surface. Despite this, these results were consistent with similar functionalizations presented in the literature¹¹ and also comparable to the contact angles measured on the CB modified SiNP surfaces prepared by QCM-D, indicating the successful generation of hydrophilic, zwitterionic functionalized SiNP surfaces.

C. CB functionalization of SiNP dispersions

While the two methods of SiNP coating functionalization presented above offer simple approaches of preparing CB modified surfaces, in some instances it may not be possible to perform a surface based modification after the application of an SiNP film. Under these circumstances, it may be advantageous to functionalize nanosilicas prior to their deposition, allowing for single stage coating fabrication. For this reason, the reaction of CB to SiNP dispersions was also investigated, and coatings of the functionalized particles were prepared for assessment of their protein resistant properties.

Reactions of CB with 10 wt. % SiNP dispersions were initially conducted at pH 3.5 and 7.0, where pH adjusted solutions of CB were slowly added to particle dispersions. However, the addition of CB to SiNPs resulted in rapid and irreversible aggregation of particles. Under these conditions, it is possible that partial protonation of the carboxylate group resulted in loss of zwitterionic character and subsequent attraction of the positively charged quaternary ammonium group to the native negative surface of the silica particles. Additionally, acid-catalyzed carboxylic acid and silanol coupling reactions could promote further particle aggregation.

This reaction was retried with the pH of the CB solution and particles adjusted to 10 to ensure complete deprotonation of CB and stabilization of particles. Under these conditions, there was no evidence of particle aggregation upon the addition of CB. After reaction, particles were placed in dialysis tubing and dialyzed against pure H_2O to remove any unbound monomer. After several days, the particle solution turned cloudy, signaling some degree of particle association. It was observed that the solution pH had fallen to ~ 7 ; however, adjusting the pH to above or below 7 restored the solutions to a clear state, indicating that particle association was a reversible process [Fig. 5(a)].

Zeta potential measurements were conducted on particle dispersions across the pH range of 3.0–11.0 [Fig. 5(b)]. While similar

(a)

(b)

FIG. 5. Images of CB modified SiNPs at various pH, showing reversible association at neutral pH (a) and ZP measurements of SiNP + CB across pH 3.0–11.0 (b).

negative ZP values were recorded for both bare SiNP and SiNP + CB at $\text{pH} \geq 9.0$, decreasing pH resulted in a dramatic positive shift in ZP values for SiNP + CB particles. The isoelectric point was estimated to be ~ 7.5 and could explain the cloudiness (aggregation behavior) observed in this pH region. The switch to a positive potential was attributed to carboxylate protonation of CB and the resultant presentation of cationic quaternary ammonium groups.

In addition to ZP measurement, the hydrodynamic diameter of particles was measured before and after functionalization with CB. As presented in Fig. 6(a), CB functionalization resulted in an increase in hydrated particle size from 18.3 to 39.5 nm. This may be partially attributed to the CB chemistry itself and its associated hydration, although the measured increase in particle size was roughly double that of bare particles and may indicate a degree of particle association.

Particles were collected via freeze-drying for FTIR and TGA analysis. The IR spectrum of functionalized particles (SiNP + CB) was compared to the spectra of unreacted SiNPs and CB (Fig. S5 in the supplementary material³³). The CB monomer presented a

FIG. 6. Hydrodynamic diameter of particles as measured by DLS (a) and TGA of bare and CB functionalized SiNPs (b).

strong band at 1585 cm^{-1} , characteristic of the asymmetric stretching vibration of carboxylic acid salts (CO_2^-),³⁰ and the small peak at 1485 cm^{-1} was assigned to methyl stretching vibrations of the quaternary ammonium group.⁷ The IR spectrum of unmodified SiNPs was relatively featureless, presenting characteristic Si–O–Si stretch as a broad peak centered at 1060 cm^{-1} and another broad peak at 800 cm^{-1} attributed to Si–OH deformation vibrations.³⁰ Functionalization of particles with CB (SiNP + CB) resulted in a broadening of the Si–O–Si band and shifted the predominant peak to 1095 cm^{-1} due to overlap from Si–O–C stretching vibrations.

Thermogravimetric analysis of SiNP + CB by gradual heating of dried particles brought about the decomposition of the CB moiety at temperatures above $200\text{ }^{\circ}\text{C}$ [Fig. 6(b)]. From the mass loss observed, the CB grafting density was calculated to be $0.92\text{ }\mu\text{mol}/\text{m}^2$. This value was comparable to the grafting density previously achieved from sulfobetaine particle functionalization.²⁰

The use of small SiNPs and the choice to conduct reactions at basic pH may have resulted in a limited degree of particle reaction, as small particles require large silane additions due to their high surface area and basic pH conditions promote self-condensation reactions which may limit silane availability for reaction to particles.

Coatings of SiNP + CB were prepared from 4 wt. % particle dispersions adjusted to either pH 3 or pH 10. Spin-coating solutions onto PEI coated QCM sensors produced thin, uniform coatings with average contact angles of 22° and 15° for coatings prepared at pH 3 and 10, respectively. The slight increase in CA for coatings prepared at pH 3 was again thought to arise from protonation of the carboxylate group and subsequent loss of zwitterionic character.

D. Protein adsorption

Protein adsorption measurements were conducted by QCM-D to compare relative protein binding to all CB functionalized SiNP surfaces. Figure 7(a) presents the calculated mass of BSA bound to surface functionalized SiNP coatings prepared by exposure to 1 and 10 mM solutions of CB at pH 3.5, 7.0, and 9.5. All CB modified surfaces presented significant reductions in BSA adsorption compared to the unmodified SiNP control surface (one-way ANOVA, $p < 0.05$). Furthermore, increasing functionalization concentration from 1 to 10 mM brought about further reductions in BSA binding, particularly for pH 7.0 modified surfaces. The improved protein resistance observed under this functionalization condition (10 mM CB, pH 7.0) was thought to be the result of increased mass of self-assembled zwitterionic monomer on the SiNP coating surface. Comparatively, SiNP coatings functionalized with CB at pH 9.5 did not experience any dramatic improvement to protein resistance when functionalization concentration was increased from 1 to 10 mM, as the mass of covalently bound CB was comparable under both concentration conditions [Fig. 1(c)]. While the protonation of CB under acidic conditions might be expected to reduce resistance to protein binding due to loss of zwitterionic character, the pH 3.5 functionalized SiNP surfaces still presented exceptional resistance to protein adsorption. It is proposed that the acid-catalyzed polymerization reactions that lead to increased mass binding at the SiNP surface also contributed to increased steric bulk at the coating interface, where compression of hydrated polymer chains brings about a steric repulsion effect, preventing the interaction and adsorption of proteins.^{31,32}

Similar protein adsorption measurements were conducted on CB modified SiNP coatings prepared via two-step surface functionalization (AS + PL) and from solution modified silica nanoparticles (SiNP + CB) [Fig. 7(b)]. In the case of the latter, 4 wt. % dispersions of SiNP + CB were adjusted to pH 3 and 10 prior to their deposition onto gold QCM sensors to compare the effect of dispersion pH on the protein resistant properties of the resultant coatings. Significant reductions in protein binding were observed across these three CB modified SiNP surfaces compared to the unmodified SiNP control surface (one-way ANOVA, $p < 0.05$). Most notably, increasing the pH of the SiNP + CB particle dispersion to pH 10 prior to coating preparation resulted in significantly improved resistance to protein binding compared to the pH 3

FIG. 7. Mass of BSA adsorption onto CB functionalized SiNP coatings with varied concentration and pH (a). Mass of BSA adsorption onto two-step CB functionalized SiNP coatings (AS + PL) and coatings of solution modified particles (SiNP + CB) deposited at pH 3 and 10 (b). Error bars represent 95% confidence intervals about the mean. Asterisks (*) indicate statistically similar subsets.

stabilized SiNP + CB coating. As the carboxylate group of CB is a strong conjugate base, it can become protonated under low pH conditions (pH 3), resulting in a loss in zwitterionic character and ultimately compromising the protein resistant properties of the coating. Ensuring the polymer was in a zwitterionic state by preparing the coatings from suspensions adjusted to pH 10 meant that improved protein resistant behavior was observed.

The two-step functionalization method (AS + PL) imparted improved protein resistance compared to the control and comparable adsorption to SiNP + CB (pH 3) coatings. The water contact angle displayed by the AS + PL coatings was also comparable to the SiNP + CB (pH 3) coatings, potentially indicating a similar degree of zwitterionic character or a low overall grafting density

resulting in slightly inferior resistance to protein binding compared to SiNP + CB (pH 10) coatings.

IV. CONCLUSIONS

This study has demonstrated multiple methods of preparing hydrophilic, zwitterion functionalized silica nanoparticle coatings. Attachment of carboxybetaine to silica nanoparticle surfaces was monitored by QCM-D, where variation of zwitterion concentration and solution pH was found to influence the mass of covalent attachment and the mechanism of zwitterion organization. A non-aqueous route of zwitterion functionalization was also employed for the preparation of CB modified SiNPs via a two-step surface modification process. Finally, CB functionalized particles were prepared as aqueous dispersions for simple deposition as thin coatings. All methods of preparing CB functionalized SiNP films resulted in hydrophilic surfaces that presented enhanced resistance to protein adsorption. Despite loss of zwitterionic character, surface modification under acidic conditions promoted polymeric growth of CB, resulting in enhanced steric bulk at the interface and good protein resistance. The slightly basic nature of the CB chemistry meant that the surface charge of modified particles could be tuned with variation of pH and thus influence the protein resistant properties of the prepared surfaces. Overall, this study has demonstrated the versatility of zwitterion functionalized SiNPs for preparing hydrophilic surface coatings with improved resistance to protein fouling.

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