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Biofouling-Resistant Electroactive Polymer Composites for Protein-Triggered Counterion Delivery

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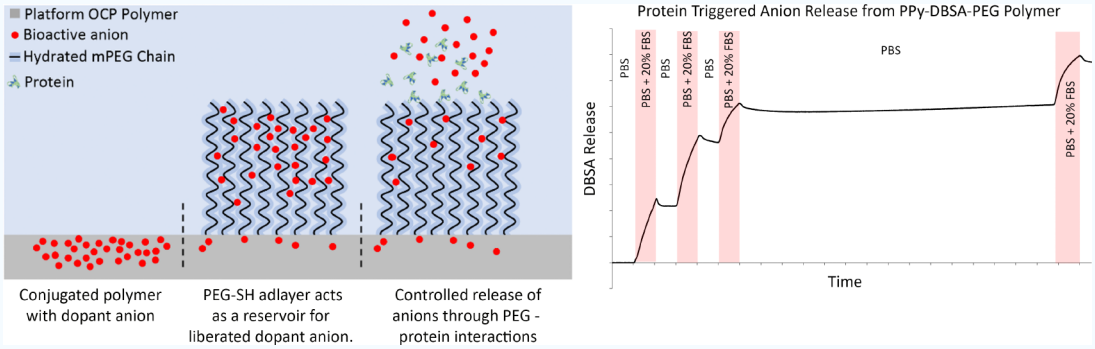

ABSTRACT: Polycationic polymers necessarily contain counterions to maintain the overall charged balance. Upon treatment of polypyrrole (PPy) films with thiol-terminated poly(ethylene glycol) (PEG), the films undergo efficient surface derivatization that reduces a portion of the film, freeing up some of the "dopant" counterions, while forming a brush-like layer. Quartz crystal microbalance (QCM) measurements show that the PEG brushes resist the adhesion of protein (fetal bovine serum (FBS)) as compared to unmodified PPy. When the dopant is polymeric dextran sulfonate, no change in the QCM resonance frequency is observed. However, when the dopant is dodecylbenzene sulfonic acid (DBSA), exposure of the film to protein causes an increase in the resonance frequency, indicating a loss of mass. X-ray photoelectron spectroscopy (XPS) and liquid chromatography–mass spectroscopy (LCMS) measurements demonstrate that the protein triggers the release of the dopant from the composite. Treatment of DBSA-doped PPy with alginate and PEG did not result in dopant release, but poly(ethyleneimine) proved to be an even more efficient trigger than FBS, revealing that an electrostatic-based mechanism is likely a primary driver for the measured DBSA release.

KEYWORDS: *polypyrrole, conducting polymer, poly(ethylene glycol), dodecylbenzene sulfonic acid, drug release, biofouling.*

1. INTRODUCTION

Inherently conducting polymers (ICPs) are a class of materials that are of interest in applications such as photovoltaics,[1] energy storage and conversion,[2] bioelectronics,[3] drug delivery,[4] and sensing,[5] among others. ICPs are typically prepared by either chemical or electrochemical oxidation or reduction reactions. During these processes, the polymer becomes charged, thus creating a need for a counterion, or "dopant", to become incorporated within the ICP matrix and balance out the newly generated polyelectrolyte.[6] This process can be used to drive the incorporation of either anions (P-doped) or cations (N-doped) into the polymer. P-doped (polycationic) polymers may incorporate simple halides, but investigators have also used a variety of organic anions that modify ICP physical properties or add new functionality. If these polymers are subsequently reduced, the dopant is no longer required. The dopant will then be expelled if possible or small charge-balancing cations will be driven into the composite if the dopant cannot be released.[6]

One application of ICPs that is especially promising is the controlled release of dopants, particularly for drug delivery.[4] A number of polymeric systems have been developed for the controlled release of drugs based on pH,[7,8] photoactivated,[9,10] or electrochemically driven[11,12] processes. Each of these approaches has limitations, the most common of which is a "burst-release" phase that releases most or all of the drug immediately after the stimuli are applied. ICPs have been investigated for incorporation and release of a variety of bioactive compounds, including anti-inflammatories,[13,14] antibiotics,[15] neurotrophins,[16,17] steroids,[18] and antipsychotics.[19]

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The release of drugs from a biomaterial surface is typically designed to act in one of two ways, either (i) the release of the drug into the neighboring biological fluid (i.e., blood, cerebrospinal fluid, endolymph) for opportunistic uptake by the surrounding cells and tissues or (ii) delivery to cells directly attached and growing on the biomaterial surface.[20] In the case of (ii), adhesion of proteins and cells may be advantageous, allowing intimate interfacing between the cells and the drug delivery interface. For cases where the target is the neighboring biological fluids and tissues (including most therapeutic applications), protein and cell adhesion is a major problem, impeding the drug's path to release and diffusion to target tissues. In the worst cases, fibrotic tissue growth can ensue, encapsulating the device and completely inhibiting drug release.

The unwanted adsorption of organics, cells, and/or microbial and macrofouling organisms to any manmade functional surface is typically termed biofouling and is a major problem that can impact any material immersed into a biologically rich environment. Such environments include blood and/or bodily fluid contacting biomedical materials and devices used for *in vitro* and *in vivo* applications, infrastructure that is submerged in marine and freshwater environments (e.g., sensors, desalination plant infrastructure, ship hull surfaces, and plumbing), and terrestrial surfaces (e.g., energy-efficient roofing, photovoltaic panel surfaces, optical sensors, building materials). A variety of approaches have been used to address this problem, including mechanical cleaning, biocide leaching materials, hydrophobic "self-cleaning" coatings, hydrophilic coatings, and others. We have previously reported that surface modification of ICP films and nanoparticles with thiol-terminated poly(ethylene glycol) (PEG) renders them resistant to the deposition of proteins, mammalian cells, bacteria, and diatoms.[21−23] These PEG species have a low free interfacial energy in aqueous environments, and the PEG chains extend in the aqueous environment to create a highly hydrophilic brush. Surface-bound PEG brushes present a dense, hydrophilic barrier at the material surface that, through water structuring and steric mechanisms, prevent the adhesion of organics, cells, and microbial organisms to the surface.[24]

In this study, we describe a new controlled counterion release ICP polymer composite platform system that presents a highly biologically antifouling interface and exhibits a new *protein-triggered* mechanism for the release of the dopant from the polymer composite. This is a system that does not have a burst-release phase. Instead, the PEG adlayer was found to mediate the diffusion of the dopant anion released from the ICP during the formation of the adlayer. The dopant was only expelled into the surrounding medium when protein interacted with the brush surface. We study the release kinetics of the dopant as a function of PEG adlayer molecular weight, identity, and loading of the dopant in the PPy film, interacting solvent and type and concentration of protein in solution. We also probed the ability of several model compounds to draw the dopant out from the PEG brush adlayer, allowing further study of the underlying mechanism/s driving dopant release.

2. MATERIALS AND METHODS

2.1. General. Pyrrole (Py) monomer was purified by distillation and stored at −18 °C. Dodecylbenzene sulfonic acid (DBSA) (289957), dextran sulfate sodium salt (DS) (D6001), phosphate-buffered saline (PBS) (5368), alginic acid (A1112), poly-(ethyleneimine) (P3143), and bovine serum albumin (BSA) (A3059) were purchased from Sigma-Aldrich (Sydney, Australia). Fetal bovine serum (FBS) was sourced from Interpath (Bovogen). Poly(ethylene glycol) methyl ether thiol (PEG-SH) of different molecular weights (1000, 5000, 20 000, and 40 000) were purchased from Sigma-Aldrich (1k) or Jenkem (5−40k MW). Deionized (DI) water was purified using a Millipore Ultrapure water purification system. All other reagents were purchased from commercial houses and used as received.

2.2. Electrochemical Polymerization of PPY Films. Electrochemical polymerization of Py was performed using a Q-Sense Electrochemistry Module (QEM 401) flow cell with a Q-Sense E4 Quartz Crystal Microbalance system (Q-Sense AB, Västra, Frölunda, Sweden). The QCM-D sensor was an A−T-cut quartz crystal with a 10 mm diameter gold electrode (QSX301) with a fundamental resonance frequency of 5 MHz (Q-Sense AB, Västra, Frölunda, Sweden). Prior to polymerization, the Au electrode surface was cleaned with piranha solution (3:1 solution of conc. sulfuric acid: 30% hydrogen peroxide) for 3 min, followed by thorough rinsing with DI water and dried under nitrogen gas. Aqueous solutions of pyrrole (Py) were prepared consisting of 0.2 M Py in deionized water with 2 $mg.mL^{-1}$ of the relevant dopant species (DS or DBSA) unless otherwise specified. Each solution was deoxygenated via bubbling with nitrogen gas for 10 min prior to use. PPy films were polymerized using an eDAQ e-corder 410 recorder and an EA163 potentiostat coupled with the QEM 401 electrochemistry module. The QEM 401 electrochemistry module consisted of a platinum counter electrode, a Ag|AgCl reference electrode, and the Au electrode on the QCM sensor as the working electrode. The aqueous Py-dopant solution was passed through the QEM 401 flow module at a rate of 60 μL/min, and once the QCM frequency (f) and dissipation (D) parameters were stable, a current density of 0.25 $mA.cm^{-2}$ was applied for 2 min for each polymer film growth (total charge of 0.03 $C.cm^{-2}$). After polymerization, the quartz electrodes supporting the polymerized films were removed from the QEM 401 flow cell, rinsed with DI water, and dried under nitrogen gas.

2.3. PEG-SH Modification of PPY Films. QCM-D sensors supporting the PPy−DS or PPy−DBSA films were placed onto a hot plate at 60 °C and left to equilibrate in air for 5 min. PEG-SH aqueous solutions for the modification of PPy films were prepared using 0.2 mM of the relevant PEG-SH MW in DI water at room temperature. PEG-SH solutions were mixed vigorously on a magnetic stirrer for 10 min. Thereafter, 200 μL of the PEG-SH solution was added to the surface of the sensor with PPy film heated to 60 °C and left to react with the film at 60 °C for 30 min. This process resulted in the water evaporating from the sensor surface, leaving behind a surface-reacted PEG adlayer and an excess thin film of unreacted PEG-SH across the polymer surface. Thereafter, the films were removed from the hot plate and rinsed vigorously under a stream of DI water to remove excess PEG, followed by drying with nitrogen gas. Unmodified PPy films were subjected to the same heating and rinsing protocol without the addition of the PEG-SH solution.

2.4. QCM-D Characterization of Solvent and Protein Interactions with PPY Films. QCM-D was used to study the interactions of solvent (DI water or PBS) and protein with PPy films with and without surface modification with PEG-SH. QCM-D sensors supporting PPy films were placed in standard Q-Sense flow modules (QFM 401) and equilibrated in the relevant solvent (PBS or DI water) at a temperature of 22 ± 0.1 °C and a flow rate of 10 μL/min until the QCM-D measurement parameters stabilized. Thereafter, the relevant solvent with added protein (5, 10, or 20% fetal bovine serum (FBS)) was introduced into the QCM-D measurement chamber at 10 μL/min for 60 min, followed by rinsing with the baseline solvent without protein until f and D measurement parameters stabilized. All experiments were performed in triplicate.

To study the background release of the dopant species in different solvents over time, sensors supporting PPy films were incubated in the QCM-D standard flow module with DI water or PBS for 12 h at a flow rate of 10 μL/min, while QCM-D measurement parameters were recorded. Thereafter, 20% FBS in the relevant solvent was introduced into the measurement chamber at 10 μL/min for 60 min, followed by

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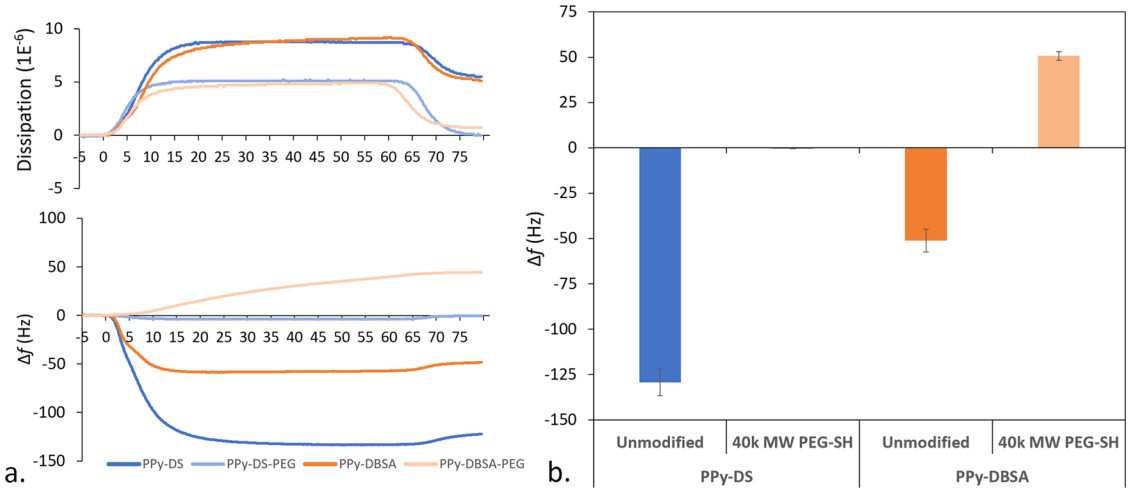

Figure 1. (a) Representative raw frequency and dissipation responses from the interaction between 20% FBS in PBS with PPy−DS and PPy−DBSA with and without modification with 40 000 MW PEG-SH. (b) Average frequency shifts from the interaction of 20% FBS in PBS with PPy−DS and PPy−DBSA with and without modification with 40 000 MW PEG-SH.

rinsing in the baseline solvent for a further 60 min until f and D measurement parameters stabilized. All experiments were performed in triplicate.

The release of the dopant species was studied over an extended period, during which the sequential addition of protein, followed by rinsing using the baseline solvent, was performed. QCM-D sensors with PPy films modified with either 5k or 40k MW PEG-SH were initially incubated in standard QCM-D flow modules in PBS for 60 min prior to introduction of protein at a flow rate of 10 μL/min. FBS (20%) was introduced in the baseline solvent at 1, 3, 5, and 19 h time points for 60 min at 10 μL/min. After each protein exposure, the baseline solvent was introduced for rinsing for at least 120 min at 10 μL/min (with this rinsing period being 14 h prior to the final protein injection at 19 h). The QCM-D measurement parameters were recorded during the entirety of the experiment. All experiments were performed in triplicate.

2.5. X-ray Photoelectron Spectroscopy (XPS) Characterization of DBSA Release from PPY−DBSA Polymers. Three PPy−DBSA films, an unmodified control and two modified with PEG-SH (40 000 MW), were prepared and equilibrated in the QCM with PBS, as described in Section 2.4. The control film and one of the PEG-modified films were flushed with PBS for an additional 60 min at a temperature of 22 ± 0.1 °C and a flow rate of 10 μL/min, while the other modified film was flushed with PBS containing 20% FBS for the same period of time. All films were then washed with DI water for 30 min. XPS data were collected on the films with a SPECS PHOIBOS 100 Analyser installed in a high-vacuum chamber with the base pressure below 10^{-8} mbar. X-ray excitation was provided by Al Kα radiation with photon energy $h\upsilon$ = 1486.6 eV (12 kV/144 W). The XPS binding energy spectra were recorded at the pass energies of 15 eV and a step width of 0.05 eV in the fixed analyzer transmission mode. To increase the signal/noise ratio, multiscans were conducted and the intensity of signals was accumulated. All spectra were referenced to carbon at 284.8 eV.

2.6. Liquid Chromatography−Mass Spectroscopy (LCMS) Characterization of DBSA Release from PPY−DBSA Polymers. PPy−DBSA films were polymerized on QCM-D sensors as described in Section 2.2 using a DBSA dopant concentration of 2 mg/mL^{-1} and surface modification with 40k MW PEG-SH performed on treatment samples, as described in Section 2.3. Unmodified control PPy−DBSA films were heated to 60 °C on a hot plate for 30 min without any further treatment as described previously. QCM-D sensors with and without 40k MW PEG-SH modification were placed in individual wells in a 12-well plate (Greiner Bio-one) in 1 mL of DI water and placed on an orbital stirrer running at ∼50 rpm at room temperature (three samples of each treatment). At time points 0, 0.5, 1, and 1.5 h, 200 μL of DI was removed from each well and placed in a small plastic vial, with the solution removed from each well replaced with fresh 200 μL of DI water. At 2 h, the DI water removed from each well was replaced with 200 μL of 100% FBS solution, resulting in a 20% FBS in DI water solution in each well. At time points 2.5, 3, 3.5, 4, and 20 h, the 200 μL of solution removed from each well was replaced with 200 μL of 20% FBS in DI water.

At the completion of the experiment, all 200 μL samples were diluted by adding 800 μL of DI water, thoroughly mixed using a vortex mixer, and then filtered through a 0.22 μm syringe filter (Econofilter PVDF, Agilent Technologies) and deposited into a sample holder prior to LCMS characterization. A single quadrupole mass spectrometer LCMS 2020 system (Shimadzu) was used for all experiments. The method parameters were as follows: mobile phase: acetonitrile/water: 90/10, isocratic. Flow rate: 0.2 mL/min. Injection volume (Direct): 5 μL. Detection: ESI mode in negative mode with single ion monitoring (SIM) at 325 (dodecylbenzenesulfonate (C18H29O3S-, m/z = 325)).[25] DL temperature: 250 °C. Nebulizing gas flow: 1.5 mL/min. Block heater temperature: 400 °C. Interface voltage: −3.5 KV.

LCMS measurements were normalized using calibration curves achieved using solutions of known DBSA concentration in DI water both with and without protein (protein concentrations used for calibration were comparable to diluted 200 μL samples containing 20% FBS (i.e., final concentration of 4% FBS)). DBSA concentrations of 80, 160, 640, 1280, 2560, and 5120 nM were used, with the resultant calibration curves available in Figure S1.

2.7. QCM-D Characterization of PPy−DBSA−PEG Films with Alginate, Polyethyleneimine, and Poly(ethylene glycol). QCM-D was used to study the interactions of alginate, poly(ethyleneimine), and poly(ethylene glycol) with PPy−DBSA films modified with either 5k MW or 40k MW PEG-SH. QCM-D sensors supporting PPy−DBSA−PEG films were initially incubated in DI water at a flow rate of 10 μL/min until f and D measurement parameters stabilized. Thereafter, alginate (0.25%), poly(ethyleneimine) (0.25 and 0.5%), or poly(ethylene glycol) (1 or 10%) in DI water was introduced into the QCM-D measurement chamber for 60 min at 10 μL/min, followed by rinsing with DI water until the f and D measurement parameters stabilized. All measurements were performed in triplicate.

3. RESULTS AND DISCUSSION

Previously, we demonstrated the ready covalent bonding of thiols to ICPs, including PPy, from aqueous and ethanolic solutions.[12,21−23,26] Consistent with our previous reports, treatment of the PPy−DBSA and PPy−DS films used in this study with aqueous solutions of PEG-SH has little effect on the

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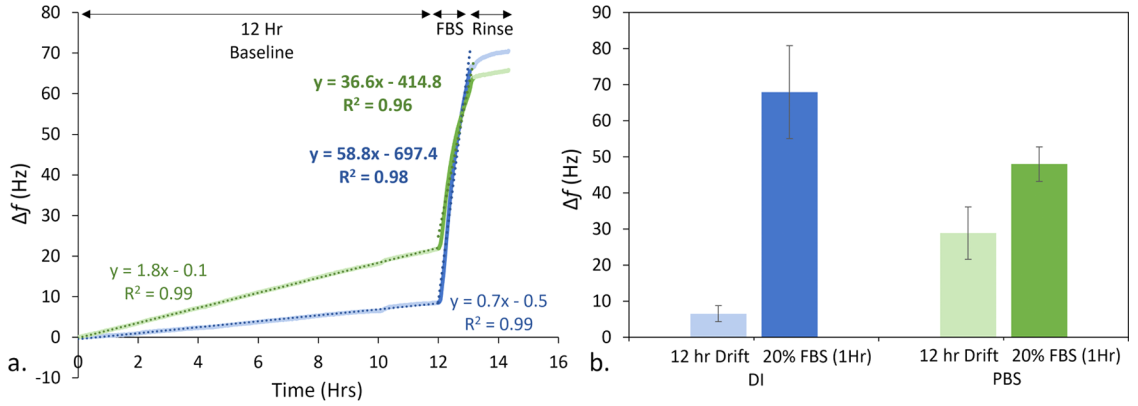

Figure 2. (a) Representative QCM-D frequency shifts (3rd overtone) for PPy−DBSA−PEG (40k MW PEG-SH) polymer films incubated in DI water (blue) or PBS (green) over a 14 h period. 20% FBS in the respective solvent was introduced at 12 h, followed by rinsing in the baseline solvent. Models were fitted to the initial baseline and 20% FBS incubation periods for each solvent type, with equations, along with the associated R^2 value, for each region presented. (b) Mean QCM-D frequency shifts for PPy−DBSA−PEG (40k MW PEG-SH) films over a 12 h incubation in the baseline solvent (DI water or PBS) and subsequent frequency response on introduction of 20% FBS into the QCM-D sample chamber. Values represent the mean from triplicate measurements, with error bars representing 95% confidence intervals around the mean.

film morphology; however, the resulting brush-like structure does affect the film interfacial properties.

QCM-D was used to evaluate the ability for the PEG-SH (40k MW)-modified PPy−DS and PPy−DBSA films to resist protein adsorption. Protein adsorption from PBS containing 20% FBS showed unmodified PPy−DS to adsorb significantly more protein (−129.3 ± 7.2 Hz) than PPy−DBSA (−51.1 ± 6.2 Hz) (Figure 1) over a 60 min incubation period. Modification with PEG-SH resulted in a reduction in protein binding to the PPy−DS film of >99% (−0.2 ± 0.2 Hz). Unexpectedly, however, in the PPy−DBSA−PEG films, a *positive* frequency shift, as opposed to a negative shift, was measured on introduction of protein into the QCM-D measurement chamber, measuring +50.7 ± 2.4 Hz after 60 min of incubation (Figure 1).

A large positive Δf (i.e., 10 s of Hz) is almost exclusively representative of a mass loss from an adlayer coupled to the quartz resonator—in this case, the PPy−DBSA−PEG film. Exceptions include measurements taken during complex and dynamic adhesion processes of microbial and mammalian cell adhesion to QCM sensor surfaces.[27−29] In our system, the positive Δf can only be attributable to protein−polymer interactions and/or the movement of water and ions between the film and the interacting solvent. Positive frequency shifts have been observed due to the dehydration of surface adlayers.[30,31] In these cases, positive frequency shifts are coupled with changes in adlayer viscoelasticity, measured as a reduction in the QCM-D dissipation factor, indicating a stiffening or rigidification of the layer as water is removed from the film. Here, the positive frequency shift commences on introduction of the protein solution and continues during the entire 60 min incubation with protein, until the positive shift levels off on rinsing with fresh PBS. This significant and irreversible change in f was coupled with a relatively minor change in D (1.0 ± 0.4), which was slightly higher, though similar, to the ΔD observed for the PPy−DS−PEG composite. We therefore propose that this positive frequency shift was unlikely to be derived from dehydration of the PPy−DBSA−PEG composite film. We have previously shown that PPy−DBSA films modified with PEG-SH from aqueous medium are highly resistant to protein adhesion,[22] and therefore we do not interpret the positive frequency shift to be correlated with protein binding to the PPy−DBSA−PEG surface. Further, there is no obvious mechanism by which protein binding to the substrate from solution would elicit such a $+\Delta f$.

An alternative hypothesis was required for the source of the "missing mass" that is lost during the PPy−DBSA−PEG interaction with the proteinaceous solution but is not seen for the PPy−DS−PEG films. During the covalent bonding of the thiol group to the carbon double bond on the PPy backbone, PPy is reduced, resulting in the liberation of the dopant anion that had been incorporated into the polymer during polymerization. If the dopant is small and mobile, it may escape from the polymer matrix and move out into the interfacial environment; however, if the dopant is large and immobile, it may be entrapped within the polymer matrix at the interface, even though it is no longer required as the polymer dopant. DBSA is a small-molecular-weight (348.48 g/mole) surfactant that has been widely used as a dopant in ICP polymerization due to good apparent polymer cytocompatibility[32,33] and the ability to modulate the surface energy of DBSA-doped ICPs using electrochemical stimulation.[34] DS is a large-molecular-weight (>500,000 g/mol), highly hydrated polysaccharide that has demonstrated anticoagulant[35] and antiviral properties[36] and has also been shown to generate cytocompatible polymers when used as a dopant in ICPs.[37,38]

We hypothesized that for the PPy−DBSA polymer, the reduction of the PPy chains at the polymer interface by covalent thiol bonding liberates the small DBSA anion from the polymer film and into the PEG layer. The PEG adlayer on the polymer film then is proposed to act as a holding reservoir for the DBSA anion, releasing the dopant from the PEG layer through the interaction with proteins at the PPy−DBSA−PEG interface. To test this hypothesis, we first examined the stability of PPy−DBSA films modified with 40k MW PEG-SH over a 12 h period using QCM-D, prior to the addition of protein (20% FBS), using either DI water or PBS as solvents. A gradual linear positive frequency was presented over the 12 h incubation without protein for both solvents (Figure 2a), with the Δf greater for PBS relative to DI water (Figure 2b). On introduction of 20% FBS at 12 h, a substantial increase in the +ve Δf was observed for all samples, with the gradient of the Δf increasing by ∼12 400 and 1995% for the DI water and PBS solvents, respectively. All changes in frequency in response

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to protein addition into the QCM-D chamber demonstrated a polynomial relationship over the 60 min incubation period, with the rate of Δf slightly decreasing over the 60 min protein incubation period, followed by a leveling off of the f shift after rinsing in the baseline solvent. The total positive f responses on protein addition were greater for the samples in DI water, relative to PBS (Figure 2b).

The positive frequency shifts that presented during these initial experiments suggested the removal of coupled mass from the PPy−DBSA−PEG sensor surface; however, the QCM-D technique is unable to determine the identity of the decoupled, or released, component. We therefore examined films modified with PEG-SH with and without exposure to FBS by X-ray photoelectron spectroscopy (XPS) and compared the data to that of an unmodified control. Figure 3 shows high-resolution scans of the S (2p) signal for the three

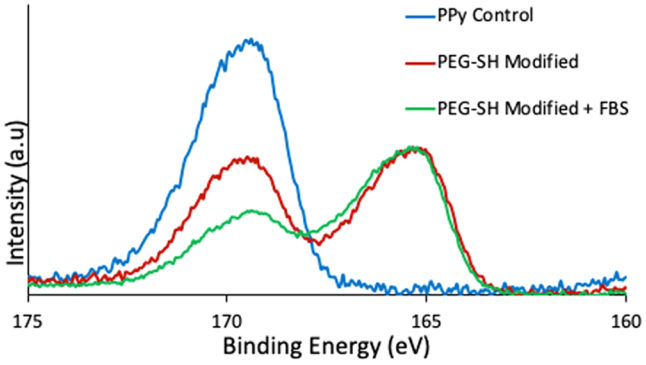

Figure 3. High-resolution S (2p) XPS data for an as-grown PPy film after modification with 40k PEG-SH and after exposing the modified film to FBS.

films. The control film shows the presence of a highly oxidized sulfur with a binding energy of *ca.* 169 eV, consistent with DBSA. Upon surface modification, a second type of sulfur appears at *ca.* 165 eV. This is consistent with a more reduced sulfide atom, and we assign it to the covalently bonded PEG-S-PPy. Since XPS is a surface-sensitive technique, the intensity of the DBSA peak in the control sample cannot be directly compared to the unmodified film, as the presence of the PEG adlayer increases the mean free path of the ejected electron and decreases the signal. Indeed, the total accumulation time for each of the modified films was twice that of the control. Exposure to FBS resulted in a sharp decrease in the DBSA signal, consistent with the proposed explanation for the frequency increase observed with QCM-D. When the sulfide signal is normalized (Figure 3), the decrease in the DBSA signal was approximately 35%.

The XPS data presented qualitative evidence that DBSA is lost from the film surface upon FBS exposure. To identify and quantify the mass of the individual components released from the film into DI water over a long (20 h) period with and without protein (20% FBS), LCMS was employed. Aliquots taken from samples immediately on incubation in DI water (time 0 h) revealed a comparable initial release of DBSA from PEG-SH (40k MW)-modified (352 ± 237 ng) and unmodified control films (399 ± 200 ng) (Figure 4). During the initial 2 h incubation in DI water only, DBSA release from both treatments remained negligible, with only a minor increase in DBSA release for both polymers over this period (598 ± 209 and 679 ± 112 ng for PPy−DBSA and PPy−DBSA−PEG, respectively). The introduction of 20% FBS in DI water resulted in a large increase in DBSA release for the PPy−DBSA−PEG polymer after 30 min (2.5 h time point), while no significant increase in DBSA release was observed for the PPy−DBSA control. DBSA release from the PPy−DBSA−PEG continued for the remainder of the experiment, eventually presenting a total release of 3436 ± 320 ng after 20 h incubation. The DBSA release profile for the initial 2 h incubation in protein was best described using a polynomial function (Figure S2). The unmodified film did not present an obvious increase in DBSA release in the presence of protein, with the final mass of DBSA release at 20 h reaching 871 ± 166 ng (Figure 4).

The Sauerbrey equation[39] can be used to estimate the mass loss from comparable PPy−DBSA−PEG polymers over a 1 h period in the presence of 20% FBS in DI water, allowing a comparison of the measurements achieved using LCMS and QCM-D (although the experimental conditions differed). The mass loss measured using QCM-D equates to 1201 ± 207 ng.cm^{-2}. Normalizing the LCMS data to the available sensor surface area, the same period of exposure to 20% FBS in DI water, equated to the measured DBSA release of 1067 ± 309 ng.cm^{-2}, closely matching the QCM-D results and confirming the positive frequency shift measured via QCM-D to originate from the liberation of DBSA from the PPy−DBSA−PEG composite polymer film. The influence of the solvent on DBSA release is less clear, with the expected moderate dehydration of the PEG chains in the PBS solution, one possible explanation for the greater DBSA release in this solvent. If the stabilization of the DBSA in the PEG layer is driven by the PEG hydration and associated water structuring, any disruption of this hydration may result in increased leakage of DBSA both with

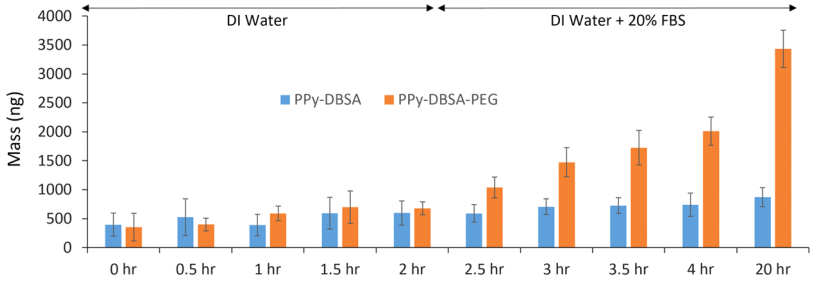

Figure 4. LCMS measurement of DBSA release from unmodified (blue) and PEG-SH (40k MW)-modified (orange) PPy−DBSA polymer films incubated first in DI water (0−2 h) and then in DI water with 20% FBS (2−20 h). Error bars representing 95% confidence intervals around the mean.

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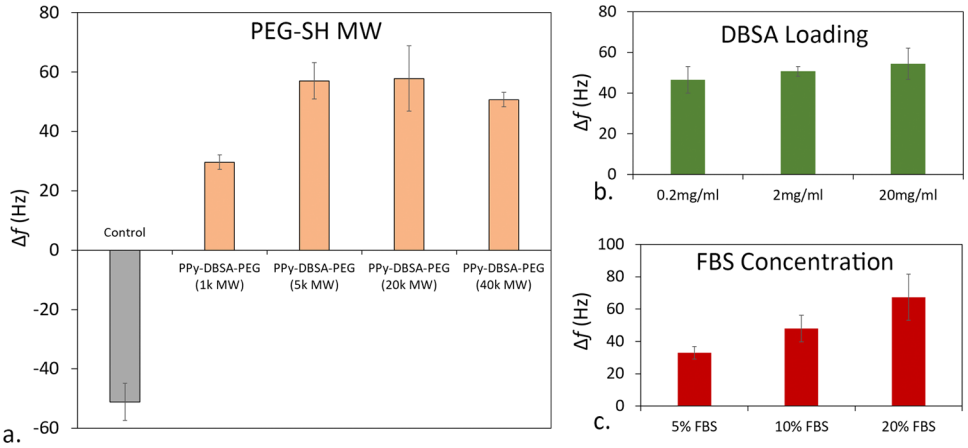

Figure 5. (a) QCM-D Δf response from the incubation of unmodified and PEG-SH-modified PPy−DBSA films in PBS with 20% FBS. (b) QCM-D Δf response from the incubation of PPy−DBSA−PEG (40k MW PEG-SH) films polymerized with different concentrations of DBSA (0.2, 2, or 20 mg/mL) in PBS with 20% FBS. (c) QCM-D Δf response for PPy−DBSA−PEG films (40k MW PEG-SH) incubated in PBS with differing concentrations of FBS (5, 10, or 20% FBS). Error bars represent 95% confidence intervals about the mean.

or without protein. For simplicity, all experiments hereafter have been performed using PBS as the solvent.

To probe the influence of PEG-SH MW on DBSA release, we modified PPy−DBSA polymers with a range of different PEG-SH MWs (1, 5, 20, and 40k) and studied DBSA release using QCM-D. A positive frequency shift was seen for PPy−DBSA modified using all PEG-SH MWs; however, the magnitude of the positive shifts varied (Figure 5a). For 1k MW PEG, the Δf was +29.6 ± 6.2 Hz, which was significantly lower than that seen for the 5k MW (+57.1 ± 6.1 Hz), 20k MW (+57.8 ± 11.0 Hz), and 40k MW (+50.7 ± 2.4 Hz) PEG-SH-modified PPy−DBSA. The corresponding dissipation shifts (ΔD) were all relatively small compared to that measured for the proteinaceous film adsorbed to the unmodified PPy−DBSA, although the shifts did increase slightly as a function of PEG-SH MW (Figure S3). This positive ΔD indicates an increase in the viscoelasticity of the PEG adlayer coupled to the movement of the DBSA out of the tightly packed PEG adlayer. The lack of cell adhesion to the PPy−DBSA−PEG films leads us to propose that protein adsorption into the PEG adlayer is minimal; however, limited protein insertion into the PEG layer may play a role in guiding the change in viscoelastic properties. The minor change in PEG adlayer properties may also be driven by ion and/or water diffusion into the film following DBSA release. Regardless, the PPy−DBSA−PEG polymers were shown to maintain their antifouling properties after several days' incubation in 20% FBS in culture media with ROSA primary skeletal myoblasts (Figure S4).

Increasing the concentration of the DBSA anion in the PPy−DBSA polymerization solution from 2 mg mL^{-1} to 20 mg mL^{-1} only resulted in a small increase in DBSA release from PPy−DBSA−PEG films modified with the 40k MW PEG-SH (Figure 5b), while reducing the DBSA concentration to 0.2 mg mL^{-1} similarly resulted in a small decrease in DBSA release, relative to polymers polymerized with 2 mg mL^{-1} DBSA. Varying the concentration of the dopant anion in the polymerization solution has previously been shown to not enhance the doping of the PPy, rather simply increasing the incorporation of the anion within the polymer matrix.[40]

Our model proposes that the anion is primarily liberated through the reduction of PPy through thiol binding, and therefore, the mass of anion release should be comparable for all dopant anion concentrations. We have previously shown that the smaller the PEG-SH MW, the greater the number of thiol coupling events are observed, presumably because the smaller chains allow greater access to the polymer surface due to decreased steric congestion.[21] This therefore should result in greater DBSA release into the PEG adlayer space. However, this was not observed here, possibly due to the poorer retention of liberated DBSA in PEG adlayers composed of smaller MW chains, resulting in anion loss during rinsing.

The concentration of FBS in solution was also shown to directly influence the amount of DBSA release from the PEG-SH-modified PPy−DBSA films, with FBS concentrations of 5, 10, and 20% FBS in PBS demonstrating a Δf of +33 ± 4 Hz, +48 ± 8 Hz, and +67 ± 14 Hz after 60 min of incubation, respectively (Figure 5c).

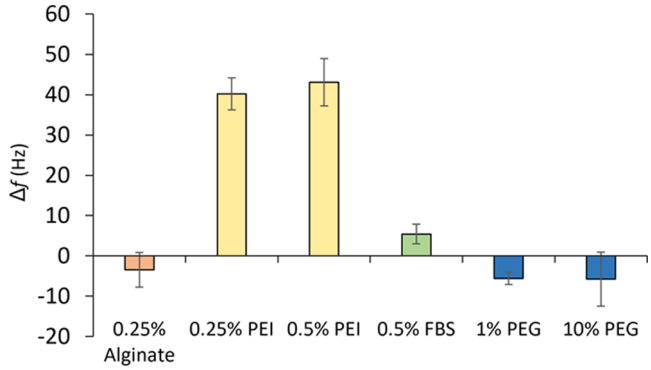

Figure 6. Average QCM-D Δf response for PPy−DBSA−PEG (40k MW PEG-SH) films after 60 min of incubation in PBS with different polymers, including alginate, poly(ethylenimine) (PEI), FBS, and poly(ethylene glycol) (PEG). Error bars represent 95% confidence intervals around the mean.

To further probe the nature of the mechanism through which protein interacts with the PPy−DBSA−PEG composite polymer to release DBSA, we replaced the FBS with polymers of differing properties. Alginate and poly(ethylenimine) (PEI) were chosen for their opposing molecular charges (negative and positive, respectively), while PEG was chosen because we were interested in understanding the capacity for PEG in solution to interact with the DBSA contained within the

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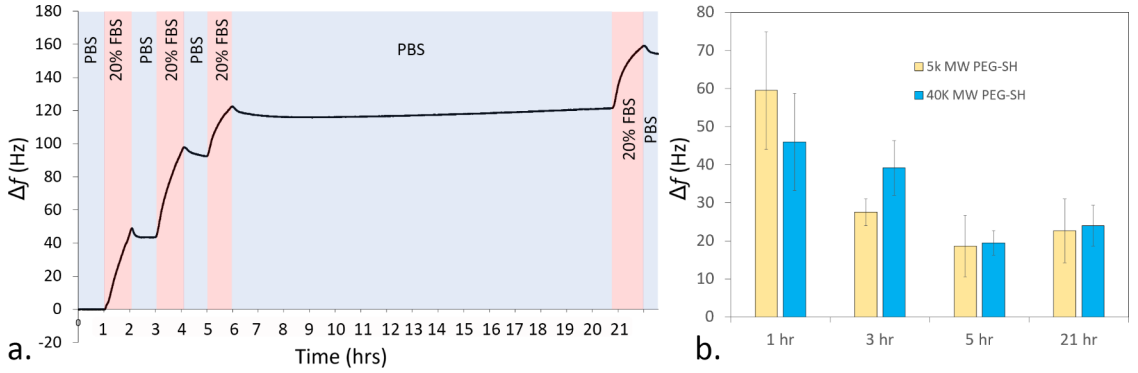

Figure 7. (a) Representative *f* response for the incubation of PPy−DBSA−PEG (5k MW PEG-SH) in PBS, with sequential exposure to 20% FBS, followed by rinsing with PBS, over a 22 h period. (b) Mean Δ*f* for the exposure of PPy−DBSA−PEG (5k or 40k MW PEG-SH) to 20% FBS at 1, 3, 5, and 21 h after initial incubation in PBS. Error bars represent 95% confidence intervals around the mean.

surface-immobilized PEG reservoir. Incubation with 0.25% alginate resulted in only a minor negative frequency shift, indicating minor adsorption to the PPy−DBSA−PEG film; however, no evidence of DBSA removal was observed (Figure 6). The failure to liberate DBSA from the film is likely due to the lack of electrostatic interactions between the alginate and DBSA, with both being negatively charged under the experimental conditions. On the other hand, PEI demonstrated a very large positive frequency shift, with 0.5% PEI producing a positive Δ*f* almost 8 times greater than that of FBS at comparable concentrations. PEI is a highly cationic polymer that has been shown to readily interact with and adsorb various anionic compounds. We propose that the efficacy of PEI in interacting with and releasing DBSA from the PPy−DBSA−PEG polymer is likely driven by electrostatic interactions between the DBSA and PEI, allowing the PEI to draw out and remove the anion from the PEG layer.

Poly(ethylene glycol) is a highly hydrated polymer formed from repeating ethylene glycol units and typically presents a neutral or slightly negative charge, depending on solution pH and terminal functionality. We were interested in understanding whether soluble PEG chains interacting with the PPy−DBSA−PEG film may interact with and draw the DBSA anions from the tightly packed surface-immobilized PEG layer. As illustrated in Figure 6, only negative frequency responses were produced on incubation in 1 and 10% PEG. Once again, the lack of electrostatic attraction between the PEG and the DBSA is the likely determinate for the lack of removal of DBSA from the film. Furthermore, the simple mechanical disturbance of the alginate and PEG-containing solutions on the surface of the PPy−DBSA−PEG films failed to result in the release of DBSA from the film (positive frequency shift), thus providing evidence that mechanical disturbance of the PEG adlayer on the PPy−DBSA film alone is insufficient to release the DBSA anions contained therein.

The sequential exposure of PPy−DBSA−PEG composite polymers to 20% FBS in PBS, followed by PBS rinsing, illustrated the capacity for the DBSA release to be turned on and off in response to the presence/absence of protein in solution. Figure 7a illustrates a representative *f* response for the exposure of PPy−DBSA−PEG (5k MW PEG-SH) to sequential 20% FBS in PBS/PBS rinsing steps over 22 h. On the introduction of 20% FBS at each relevant time point, an immediate positive *f* shift is presented for the entire 60 min exposure. However, on rinsing with PBS without protein, an instant leveling of the *f* response is presented, indicating the cessation of DBSA release from the composite polymer. These responses remain consistent for each protein exposure/rinsing cycle over the 21 h experiment. Total DBSA release was greatest for the first protein exposure for both 5k and 40k MW PEG-SH (1 h), with DBSA release reduced at each of the following two protein exposure periods (3 and 5 h) (Figure 7b). DBSA release at 21 h was comparable to that seen at 5 h for both the 5k and 40k MW PEG-SH-modified polymers.

4. CONCLUSIONS

The results presented here demonstrate that polymer brushes may be used to control the release of small anions from a polymer composite. A better understanding of the structure of PEG layers and their disruption by proteins may allow for "smart" materials that release antibiotics, anti-inflammatory compounds, and other drugs in response to contact by particular biological species. Future work in this area will require experimentation with different polymer substrates, entrapped small molecules, and new polymer layers, as well as computational simulations of the composites to help guide the design of functional systems.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsapm.1c01082.

LCMS data used for the calibration of DBSA concentration with associated line fitting and R^2 value; light microscopy images of ROSA primary myoblast cells on unmodified and PEG-SH-modified PPy−DBSA films; LCMS data for the release of DBSA (converted to ng release) from PPy−DBSA−PEG films over a 2 h period on exposure to 20% FBS in DI water (with line fitting and R^2 value); and representative raw QCM-D measurement parameter shifts from the 3rd, 5th, and 7th overtones for DBSA release from PPy−DBSA modified with different MW PEG-SH (PDF)

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Author Contributions

P.M., B.M., and T.W.H. designed the study. P.M., R.W., A.K., D.T., T.W.H., and P.H. performed experiments and undertook data analysis. P.M. and T.W.H. supervised the study. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS

PPy, polypyrrole; DBSA, dodecylbenzene sulfonic acid; PEG-SH, poly(ethylene glycol) thiol; FBS, fetal bovine serum; LCMS, liquid chromatography−mass spectroscopy

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