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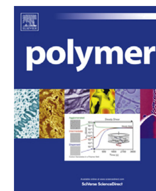
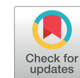


Porous star-star polyelectrolyte multilayers for protein binding



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ABSTRACT

This work employs layer-by-layer adsorption of star-poly[2-(dimethylamino)ethyl methacrylate] (star-PDMAEMA) and star-poly(acrylic acid) (star-PAA) to create porous coatings that bind the equivalent of many monolayers of lysozyme. AFM images of (star-PDMAEMA/star-PAA)$_n$ films on gold-coated wafers reveal pore diameters ranging from 100–550 nm, depending on the number of arms in the polymer and the number of bilayers, n, in the films. (Star-PDMAEMA/star-PAA)$_n$ coatings on Au-coated wafers bind the equivalent of up to 150 nm of lysozyme, and binding is greater for films containing star-PAA with 3, rather than 4, arms. Deposition of star-PAA/star-PDMAEMA films in porous hydroxylated nylon gives membranes that bind proteins via ion-exchange interactions. Such membranes capture as much as 120 mg of lysozyme per cm^3 of membrane (equilibrium binding capacity), which is more than twice the capacity of commercial ion-exchange membranes, but films are unstable at neutral and high pH.

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1. Introduction

Layer-by-layer (LBL) assembly is a versatile method for fabrication of nm- [1,2] to μm-thick [3,4] coatings with a range of compositions. Tailoring of the composition and permeabilities of these films makes them attractive as antifogging coatings [5], selective skins of membranes [6,7], drug delivery vesicles [8,9], and thin layers that capture specific proteins [10,11]. Most studies of functional polyelectrolyte multilayers (PEMs) employed linear polyelectrolytes, but recent papers examined LBL adsorption of star polymers for potential applications in fouling resistance [12] and drug release [13]. The star polymer architecture causes differences in film rheological and mechanical properties compared to PEMs prepared with corresponding linear polyelectrolytes [14]. In applications outside of LBL films, star polymers are promising candidates for use in drug and gene delivery [15–18], magnetic resonance imaging [19,20] and energy storage [21].

This work employs star polyelectrolytes to create porous or highly swollen PEMs that rapidly capture protein for potential applications such as protein purification [10] or sensing [22]. Compared to multilayer films made from linear polymers, PEMs with star polymers should show less chain entanglement to facilitate protein permeation [23]. Previous efforts to create porous PEMs employed post-deposition solvent etching [24] and UV [25], thermal [26,27], acid [24,28,29], or salt [30,31] treatments. Nanoporous films can also form through self-assembly of building blocks such as charged silica particles [32,33], block copolymers and micelles [29,34,35]. Of particular importance to this work, Hammond et al. showed that post-deposition acid treatment of star-poly[2-(dimethylamino)ethyl methacrylate] (star-PDMAEMA)/star-poly(-acrylic acid) (star-PAA) LBL films leads to porous surfaces [24]. Guo et al. found that LBL films of star-PDMAEMA/poly(sodium 4-styrenesulfonate) grow exponentially with the number of layers, and thickness is a function of star polymer arm length and the number of arms [36]. The number of star polymer arms also affects film morphological changes after acid treatment. Tsukruk et al. used star-PDMAEMA and star-PAA to construct LBL films and study pH-controlled film growth [23]. Moreover, Connal et al. showed that thin films assembled with star-PAA and linear poly(allylamine hydrochloride) (PAH) show pH-responsive reversible morphological transitions [37]. Although these studies discuss the surface morphologies of films containing star polymers, they do not demonstrate direct formation of porous films upon deposition of star polyelectrolytes or examine the adsorption of biomacromolecules.

In this study, we fabricate porous star-PDMAEMA/star-PAA multilayer coatings without post treatment, and utilize these

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porous coatings for rapid protein capture on flat substrates and in porous membranes. Both pore size in dry films and protein binding vary with the number of arms on the star polymers and the number of layers in the film, and pore diameters in dry films reach 550 nm. Remarkably, films bind as much as 50 monolayers of lysozyme, and the equilibrium binding capacities of star polymer-modified membranes are as much as 120 mg of lysozyme per cm^3 of membrane at pH 5.4.

2. Experimental

2.1. Materials

Hydroxylated nylon (LoProdyne LP, Pall, 1.2 μm pore size, 110 μm thick) membranes and Au-coated Si wafers (200 nm of sputtered Au on 20 nm of Cr on Si (100) wafers, coating was performed by LGA Thin Films, Santa Clara, CA) were cleaned in a UV/O_3 chamber for 10 min prior to use. 3-Mercaptopropionic acid (MPA, 99%) (Aldrich), coomassie protein assay reagent (Thermo Scientific), and lysozyme (Sigma-Aldrich) from chicken egg white were used as received. Buffers were prepared using analytical grade chemicals and deionized water (Milli-Q, 18.2 MΩ cm).

2.2. Synthesis of star-polyelectrolytes

Star-PDMAEMA and star-PAA were synthesized by atom transfer radical polymerization (ATRP) in a core-first approach following a modified literature procedure [38–41]. The supplementary content describes the polyelectrolyte synthesis in detail and also provides NMR spectra of the polymers (Fig. S9 and S11). (Figure numbers beginning with “S” refer to the supplementary content.) Cationic and anionic star polymers are abbreviated as star-PDMAEMA-X and star-PAA-X, where “X” is the number of arms (3 or 4) in each star polymer.

2.3. LBL assembly of star polymers at low pH and constant ionic strength

Aqueous solutions of 0.01 M star-PDMAEMA-X or star-PAA-X (polymer concentrations are given with respect to the repeating unit assuming repeat unit molecular masses of PAA = 76.1 g/mol and PDMAEMA = 157 g/mol) were prepared in deionized water containing 0.5 M NaCl. The pH of each solution was adjusted with the addition of 1 M HCl or 1 M NaOH. A monolayer with -COOH groups was adsorbed on Au-coated Si wafers (24 mm × 11 mm) by immersing the wafer in 5 mM MPA in ethanol for 12 h, rinsing with ethanol, and drying with N_2. A star-PDMAEMA-X layer was deposited by immersion of an MPA-coated substrate in a 0.01 M solution of star-PDMAEMA-X for 5 min, where solution pH values were adjusted to 3.0 prior to adsorption (with 0.5 M NaCl in solutions). After washing with 1 mM HCl (no NaCl) for ~1 min and drying with N_2, the Au-MPA-(star-PDMAEMA-X) substrates were immersed in a 0.01 M star-PAA solution (adjusted to the desired pH of 3.0, with 0.5 M NaCl in the solution) for 5 min and again rinsed with 1 mM HCl and dried with N_2. This polyelectrolyte adsorption process was repeated to obtain the desired number of multilayers, n, in Au-MPA-(star-PDMAEMA-X/star-PAA-X)$_n$ films.

2.4. Characterization of initiators, monomers, polymers, and (star-PDMAEMA-X/star-PAA-X)$_n$ films

The supplementary content provides detailed characterization of initiators, monomers and polymers. Diameters of star polymers were determined using light-scattering from solutions containing 1 mg/mL of polymers. Measurements were performed on a Zetasizer Nano ZS apparatus (Malvern, U.K.). The thicknesses of PEMs were determined with a rotating analyzer spectroscopic ellipsometer (model M-44, J. A. Woollam) using WVASE32 software. Both refractive index and thickness were fitting parameters. A Cauchy model, $n(\lambda) = A_n + \frac{B_n}{\lambda^2} + \frac{C_n}{\lambda^4}$ was employed to fit the film refractive index as a function of wavelength. Reflectance Fourier Transform Infrared (reflectance FTIR) spectra of films were obtained with a Thermo Nicolet 6700 FTIR spectrometer that contained a mercury-cadmium telluride detector and a PIKE grazing angle (80°) attachment (p-polarization). Typically, 128 scans were collected for each spectrum. The AFM morphology images (Cypher™ atomic force microscope) of (star-PDMAEMA-X/star-PAA-X)$_n$ films on Au-coated wafers were recorded in tapping mode (amplitude ratio = 0.90–0.99) using a silicon nitride tip. AFM images are shown in height mode without any image processing except flattening. Scanning rates were between 1.0 and 2.0 Hz.

2.5. Lysozyme binding on flat substrates and in porous membranes

Substrates coated with (star-PDMAEMA-X/star-PAA-X)$_n$ were immersed for 24 h in a solution containing 0.1 mg/mL of lysozyme in 20 mM phosphate buffer (pH 5.4). Because these films are not stable in pH 7.4 phosphate buffer (as shown from IR spectra and ellipsometry data), we chose pH 5.4 buffer for protein binding studies. After protein sorption, each substrate was separately rinsed with 10 mL of washing buffer (20 mM phosphate buffer at pH 5.4) and dried with N_2. The amount of lysozyme binding was determined by reflectance FTIR spectroscopy and reported with respect to the equivalent thickness of spin-coated lysozyme which would give a similar absorbance. The equivalent thickness d can be calculated from the difference of absorbance (ΔA) at 1680 cm^{-1} (amide band I of lysozyme) before and after binding lysozyme, using the equation d(nm) = ΔA/0.0017 [42].

Membranes with 3.1 cm^2 of exposed external surface area (110 μm thickness, 35 μL volume) were cleaned for 10 min with UV/ozone and placed in a homemade Teflon holder. Subsequently, a 20 mL solution containing 0.01 M star-PAA-X and 0.5 M NaCl at pH 3.0 was circulated through the membrane for 30 min at a flow rate of 1 mL/min using a peristaltic pump. Additional star-PDMAEMA-X or star-PAA-X layers were deposited in the same manner. After deposition of each polyelectrolyte layer, 20 mL of 1 mM HCl was passed through the membrane. Lysozyme binding capacities of porous membranes were calculated using breakthrough curves obtained by passing 0.3 or 1 mg/mL protein solutions (in 20 mM phosphate buffer at pH 5.4) through membranes. The solution flow rate was 1 mL/min (29 membrane volumes/min), and Bradford assays (using calibration with lysozyme) were employed to quantify the concentrations of proteins in consecutive aliquots of membrane effluent. Integration of the differences between the feed concentration and the effluent concentrations yields the equilibrium binding capacity of membrane, which we report as the total adsorbed lysozyme mass per unit volume of membrane (including porous and nonporous regions). Elution of captured protein occurred during passage of 0.5 M NaCl in 20 mM phosphate buffer at pH 5.4 (four 2-mL aliquots at 1 mL/min, most of the protein eluted in the first two aliquots).

3. Results and discussion

3.1. LBL assembly of porous star polymer films

This work aims to use LBL adsorption of star-PAA (Fig. 1(a)) and star-PDMAEMA (Fig. 1(b)) to create porous or highly swollen films to enhance the rate and amount of protein binding in platforms such as porous membranes. Synthesis of cationic star-PDMAEMA

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