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Surfaces for Competitive Selective Bacterial Capture from Protein Solutions

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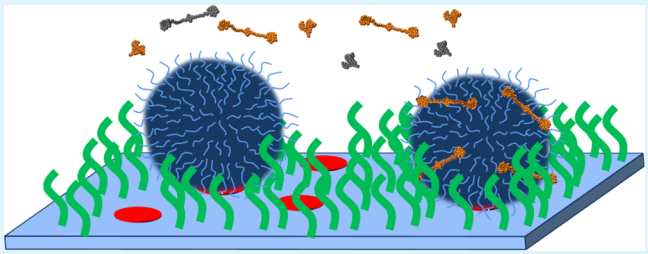
ABSTRACT: Active surfaces that form the basis for bacterial sensors for threat detection, food safety, or certain diagnostic applications rely on bacterial adhesion. However, bacteria capture from complex fluids on the active surfaces can be reduced by the competing adsorption of proteins and other large molecules. Such adsorption can also interfere with device performance. As a result, multiple upstream processing steps are frequently employed to separate macromolecules from any cells, which remain in the buffer. Here, we present an economical approach to capture bacteria, without competitive adsorption by proteins, on engineered surfaces that do not employ biomolecular recognition, antibodies, or other molecules with engineered sequences. The surfaces are based on polyethylene glycol (PEG) brushes that, on their own, repel both proteins and bacteria. These PEG brushes backfill the surface around sparsely adsorbed cationic polymer coils (here, poly-L-lysine (PLL)). The PLL coils are effectively embedded within the brush and produce locally cationic nanoscale regions that attract negatively charged regions of proteins or cells against the steric background repulsion from the PEG brush. By carefully designing the surfaces to include just enough PLL to capture bacteria, but not enough to capture proteins, we achieve sharp selectivity where *S. aureus* is captured from albumin- or fibrinogen-containing solutions, but free albumin or fibrinogen molecules are rejected from the surface. Bacterial adhesion on these surfaces is not reduced by competitive protein adsorption, in contrast to performance of more uniformly cationic surfaces. Also, protein adsorption to the bacteria does not interfere with capture, at least for the case of *S. aureus*, to which fibrinogen binds through a specific receptor.



KEYWORDS: *competitive bacterial capture, selective, bacterial adhesion, bacterial coadsorption, protein-tolerant, nonfouling*

INTRODUCTION

Much research in the biomaterials field focuses on the development of bacterially nonadhesive coatings, to prevent biofouling and biofilm formation.[1] However, when the surface is part of a diagnostic device or sensor (for instance, within food packaging or a continuous online bacteria threat sensor), bacterial capture is the goal. The tendency for bacteria to stick to surfaces,[2,3] and the large difficulty in preventing this, might lead one to expect that the design of surfaces that target bacteria adhesion would be trivial. However, the design of bacteria-targeting surfaces can be challenging. The challenges include the capture and detection of small bacterial numbers from dilute suspensions and solutions, and the ability to capture bacteria in a complex environment. Solutions of interest (body fluids, food, etc.) may contain species that compete with bacteria for surface sites, lowering capture efficiency and detection sensitivity,[4−8] and obstructing antimicrobial activity for contact-type surfaces.

One way to adhesively target specific bacteria is to employ surface-immobilized antibodies against the bacteria of interest.[9−13] Antibodies are well-suited to diagnostics where key bacteria strains are distinguished by unique surface markers. However, antibodies can be expensive and, because their targeting is highly specific, they miss nontargeted bacterial types.[14] For this reason, some technologies employ combinations of antibodies to target multiple pathogens.[15,16] Other clever alternatives include the use of highly specific aptamers[17] or arrays of lectin-targeting carbohydrate hydrogel microarrays.[18] When surfaces target specific bacteria or bacterial combinations through this biomolecular targeting approach, the surface must also be coated with a nonadhesive species such as polyethylene glycol (PEG) or albumin, to prevent nonspecific adhesion of nontargeted molecules and cells. Therefore, the specificity of these surfaces is limited by that of the passivating layer. Further the immobilized biomolecular capture agent (antibody, aptamer, etc.) must be presented in a way that avoids obstruction by the nonfouling treatment. Often tethering the biomolecular capture agents forward of the nonfouling surface has been a useful strategy.[19,20]

Sensors for bacterial exposure and contamination are subject to different technical and economic constraints, compared with functional surfaces within diagnostic technologies. The former must be inexpensive, robust, and broadly nonfouling. Applications may require faster response times but relax the

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need to identify specific strains: rough identification of bacteria type may be sufficient. These requirements drive the exploration of new surfaces that capture bacteria through means other than highly specific biomolecular recognition, but still maintain some level of selectivity to distinguish bacteria from competing macromolecular species, for instance, avoiding fouling by proteins. While numerous studies employ uniform model surfaces to quantify the nonspecific surface forces (hydrophobic, hydrogen bonding, electrostatic, van der Waals) dominating the adhesion of different bacterial types,[21−24] we are unaware of studies that address the issue of competitive adsorption between proteins and bacterial species. We also do not find, in the current literature, descriptions of surfaces capable of adhering bacteria (without use of antibodies or aptamers) in the presence of protein solutions without directly adhering the proteins themselves. Selective bacterial adhesion, relative to proteins, is challenging, because the electrostatic, hydrophobic, van der Waals, and hydrogen bonding interactions that drive bacterial adhesion[21−24] do the same for most proteins.[25,26]

The current study lays the foundation for the design and fabrication of surfaces that competitively capture bacteria from complex solutions without fouling by other species and without reliance on molecular recognition by immobilized antibodies or other biomolecular fragments.

This paper focuses on competitive bacteria adhesion on engineered surfaces that we previously characterized extensively and studied in other contexts.[27−30] The surfaces are based on classical nonfouling PEG brushes that contain randomly and sparsely distributed nanoscopic (5−15 nm) embedded adhesive species. While these adhesive "stickers" approach the sizes of proteins, they do not undergo biomolecular recognition. The stickers are simple cationic polyelectrolytes[27] or functionalized nanoparticles[31] that attract negatively charged regions on approaching objects. In this work, the adhesive surface features are adsorbed coils of poly-L-lysine (PLL), with free solution diameters in the range 5−8 nm from dynamic light scattering.[27] The PLL coils are randomly distributed on a supporting surface and usually at overall loadings corresponding to average coil spacing greater than the average coil diameters. As a result, individual PLL molecules are isolated, randomly distributed, and interact as discrete surface features.[27,28] Key to the design of these surfaces is (1) the weak binding of individual "stickers" potentially facilitating the removal of targeted species, (2) the embedding of the adhesive stickers within the brush so that steric repulsion of approaching species competes with attractive interactions to produce selectivity, and (3) the entirely nonadhesive character (to proteins and bacteria) of the PEG brush without embedded species, achieved through appropriate choice of molecular and interfacial brush architectures. Indeed, the selectivity of brushy surfaces containing embedded stickers is limited by the quality and nonfouling character of the PEG brushes upon which they are based. The brushes we employ have been optimized to eliminate protein[32−34] and bacterial adhesion on their own.[35] Prior studies established a multivalent binding mechanism for brushes containing embedded cationic coils, where proteins bridged between adhesive surface patches[28] and where, separately, bacteria could adhere to the same patches as though they were flaws in the brush.[29] Statistical arguments were made for the specific surface designs where steric repulsion was overcome by adhesion to the isolated PLL patches. Also worth emphasizing, the strategy to embed adhesive functionality within the brush is a departure from the design of surfaces with biomolecular fragments that accomplish molecular recognition. These biomolecular fragments usually target most effectively when tethered forward of the PEG brush,[19,20] which simply acts to passivate the remaining surface. Our design relies on competition between steric repulsions and localized attractions, which is achieved by burying the adhesive functionality within the brush.

This paper first describes a strategy for the design of surfaces that capture bacteria from protein solutions. The approach is then demonstrated using a particular bacterium, *S. aureus*, to establish the feasibility and the range of selectivity that can be achieved. Finally, the paper discusses the extension from *S. aureus* to other bacterial species, and outlines specific requirements and behaviors, which could be implemented as predictive screens for the performance of materials with other species.

S. aureus is chosen as a model bacterium, because it possesses binding receptors for fibrinogen.[36] This work therefore examines the competitive adsorption of *S. aureus* in the presence of fibrinogen, and may adsorb onto both *S. aureus* and the collector. This behavior is compared to *S. aureus* capture from albumin solutions, where albumin competitively adsorbs onto the collecting surface but not to *S. aureus* itself.

EXPERIMENTAL DESCRIPTION

Materials. Experiments were conducted in pH 7.4 phosphate buffer unless otherwise noted. This was made from 0.002 M KH_2PO_4 and 0.008 M Na_2HPO_4.

The adhesive patches were made of poly-L-lysine (PLL) having a nominal molecular weight of 20 000 g/mol from Sigma. At the ionic conditions in our studies, the PLL is configured as a random coil and, when adsorbed to silica, the PLL coils form flat patches[37] that are too flat to be detected by dynamic light scattering (DLS).[38]

The same PLL was also used as the central backbone of the PLL−PEG graft co-polymer that formed the basis for the PEG brush. In the graft co-polymer, roughly 30% of the PLL's amine groups were functionalized with PEG side chains having a molecular weight of 5000 g/mol. When adsorbed to a negative surface such as silica or another metal oxide, the PLL backbone physisorbs to the substrate and the PEG coils protrude into solution to produce the brush. This approach to create PEG brushes followed that developed and extensively studied in the Textor lab.[32,33] The electrostatic anchoring of the PEG tethers is robust to protein challenge over a broad ionic strength range, beyond the conditions employed here. The method can be modified to permanently anchor the adsorbed co-polymers, although we did not find it necessary here, as the brushes and adsorbed PLL patches were extremely stable. The choice of 30% PLL functionalization corresponds to the copolymer composition forming a brush with complete resistance to bioadhesion within detectible limits.

The functionalization reaction of the PLL by the PEG chains employed the *N*-hydroxysuccinimidyl ester of methoxypoly(ethylene glycol) acetic acid (Laysan Bio Inc.), as described.[27] The reaction product was purified by dialysis against phosphate buffer for 24 h and then against deionized (DI) water for 24 h. The amount of PLL functionalization was determined by ^{1}H NMR in D_2O solvent, using a Bruker 400 MHz instrument. The lysine side-chain peak ($-CH_2-N-$) at 2.909 ppm and the PEG peak ($-CH_2-CH_2-$) at 3.615 ppm were quantified to determine the amount of PLL functionalization by PEG.

Bovine serum fibrinogen (fraction I, type I-2), from Sigma−Aldrich (Catalog No. F8630-1G), and bovine serum albumin, also from Sigma−Aldrich (Catalog No. A7511), were used as received, except when fluorescently labeled. Fluorescently labeled fibrinogen or albumin was made by reacting fluorescein isothiocyanate (FITC isomer I, F2502 from Aldrich) as previously described.[39,40] The reaction product was purified by passing the protein solution through a P-6 gel column (Biorad). Fluorescent labeling density was determined by fluorescence spectroscopy: There were 1−2 fluorescein tags per

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fibrinogen molecule, or 1−1.5 labels per albumin molecule, depending on the particular labeling batch.

Bacteria. *S. aureus* (ATCC 25923) were grown in Mueller Hinton broth. The particular strain is a clinical isolate, and is widely used in standard tests of antibiotic susceptibility. The cultures were incubated aerobically overnight at 37 °C with shaking at 200 rpm. They were harvested after a total of 24 h during logarithmic growth. Proteins and other molecules were removed by centrifuging the bacterial suspension at 1000*g*, and cells were resuspended in phosphate buffer, a procedure which was conducted twice. The final bacteria concentration for adhesion studies was 5×10^5 cells/mL. Adhesion studies were conducted at room temperature but cells were stored refrigerated near 4 °C, and used within 24 h of preparation.

Flow Chambers. Studies of protein adsorption and bacterial deposition were studied in steady-shear laminar flow chambers having a slit thickness of 1 mm. Slightly different chamber designs accommodated real-time measurements of polymer or protein adsorption via Near Brewster optical reflectometry,[41] fluorescently tagged protein via total internal reflectance fluorescence (TIRF),[39] or bacterial adhesion kinetics via lateral video microscopy.[42] In the latter, the flow chamber is oriented so that gravity does not contribute or detract from the bacteria-surface interaction. Video-micrographs were analyzed using ImageJ software to determine the numbers of bacteria in each frame. Various experiments were conducted in straightforward fashion by pumping solutions through the chamber at the specified wall shear rates. Runs at conditions of interest were typically run in triplicate. Note that the limit of detection on the reflectometer is 0.01 mg/m^2, whereas, for the TIRF flow instrument, it is ∼0.03 mg/m^2 for the modestly labeled proteins in the current study. This level of precision and quantitative limit of detection for assessing protein *in situ* exceeds that detectible through fluorescence microscopy.

Surface Fabrication. PLL coils and PEG brushes were deposited from flowing buffer (having a wall shear rate of 5 s^{-1}) on microscope slides and soaked overnight in concentrated sulfuric acid to produce a ∼10 nm layer of silica on their surfaces. PLL coils were first deposited by flowing a buffered 2 or 5 ppm PLL solution (concentration chosen as needed) for a controlled amount of time (on the order of minutes) to deposit the targeted number of coils per unit area. After reinjection of flowing buffer for 5 min, a flowing buffered 100 ppm solution of PLL−PEG was introduced for ∼10 min to functionalize the remaining surface area with the PLL−PEG brush. The rate of PLL adsorption was quantified separately via near-Brewster optical microscopy, to facilitate tight control of the amount of PLL deposited via controlled PLL flow time.[27] The typical error in the PLL deposition amount is ∼0.02 mg/m^2 for the current study.

While PEG brushes containing a variety of PLL densities are presented early in the Results section, most of the paper focuses on comparing PEG brushes containing 3500 PLL chains/μm^2 with a surface containing a saturated layer of PLL and no PEG brush. Estimates for the zeta potentials of the planar surfaces were made using an spherical analogue. Silica spheres, 1 μm diameter, monodisperse from Geltech (Orlando, FL), were coated with adsorbed PLL and PLL−PEG at the same compositions as the planar surfaces. In the case of PEG brushes with 3500 PLL chains/μm^2, the zeta potential at pH 7.4 and the ionic conditions of this study was −19 ± 3 mV. For a saturated layer of PLL on silica, the zeta potential was 6 ± 3 mV. Note that a saturated PLL layer, comprising the control surface composition in this study, contains 0.4 m/mg^2 or 12 000 PLL chains/μm^2. Here, the surface is essentially a dense lawn of cationic chains where the center−center coil separation is similar to or less than the coil size itself. For these reasons, we consider the saturated surface to be relatively homogeneous in electrostatic character on the length scales of interest. By contrast, with 3500 coils/μm^2, the coils separation is 17 nm, which exceeds the coil size and produces, on average, isolated coils.

Fluorescence Microscopy. Fluorescence microscopy images were obtained on a Nikon Diaphot 300 inverted fluorescence microscope employing a Plan Fluor 40× objective and a fluorescein filter cube. Images were obtained using a CoolSnap CCD camera.

RESULTS

Design Strategy Based on Capture/Adsorption from Single-Species Solutions. The design strategy for surfaces that capture bacteria from solutions of potentially adsorbing proteins starts with a comparison of the single-component adsorption behavior of the individual species. When *S. aureus* or proteins are individually dissolved in phosphate buffer, their capture on engineered surfaces is dependent largely on the surface design, as summarized in Figure 1, for PEG brushes

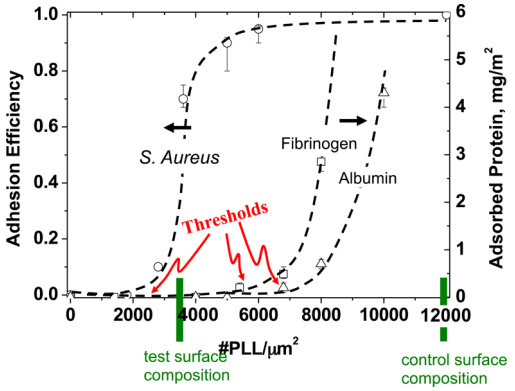

Figure 1. Capture of *S. aureus* (left axis) or proteins (right axis) from single-component buffered solutions with 5×10^5 cells/mL or 100 ppm protein, respectively. The surface density of PLL is varied on the on the *x*-axis and all surfaces are backfilled with a PLL−PEG brush where the PEG chains are 5000 g/mol. The pH is 7.4 and the ionic strength is 0.026M. Data represent the average of at least three runs while error bars show the range of the data.

containing increasing amounts of embedded PLL on the *x*-axis. Here, the *S. aureus* capture efficiency, on the left axis, is defined as the observed capture rate normalized by the transport-limited rate. The bacteria capture rates are determined, for each run, from the portion of the run where the numbers of captured bacteria are low and, accordingly, bacteria accumulation is linear in time. In this limit, which persists for at least the first several minutes of each run, hydrodynamic effects from bacteria already on the surface do not appreciably influence capture of subsequent bacteria. We choose this rate metric for bacterial capture because the ultimate amount of bacteria adhered is not meaningful: Ultimate coverages, which are substantially dependent on flow rate and bacterial shape, because of a hydrodynamic shadowing effect,[43,44] do not provide information about adhesion in a straightforward fashion. The rate metric can provide insight into the bacteria−surface interaction potential through classical Smoluchowski theory.

For the case of protein adsorption (on the right axis of Figure 1), the adsorbed mass at 30 min is chosen as a metric. The coverage at 30 min describes the coverage near the saturation value. Any additional slow adsorption is highly system- and history-dependent and is irrelevant to the initial capture that determines the competitive bacteria and proteins adhesion that sets up the initial surface composition. This near-saturation coverage is meaningful for fibrinogen and allows a comparison with literature reports of fibrinogen coverage. The differences in the units for protein and bacteria capture do not pose a problem for the material design strategy described below, and are appropriate given the different roles of hydrodynamics in bacteria or protein capture.

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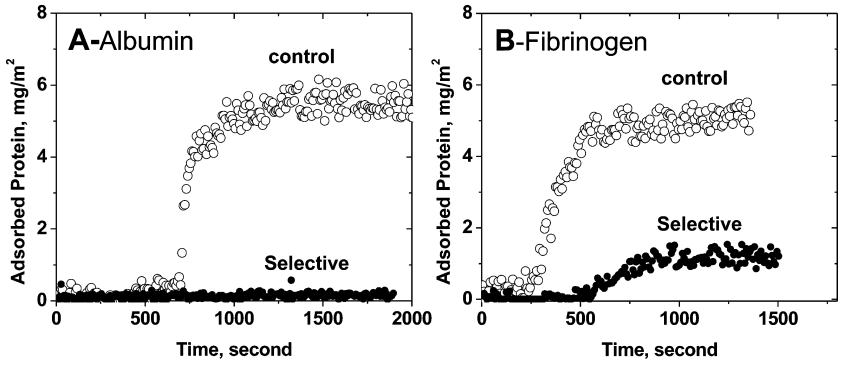

Figure 2. Interfacial accumulation of labeled protein from binary protein−*S. aureus* mixtures, measured by TIRF: (A) albumin adsorption from bacteria plus 2000 ppm albumin and (B) fibrinogen accumulation from bacteria plus 200 ppm fibrinogen. The bacteria concentration is 5×10^5 cells/mL. The selective surface contains 3500 PLL coils/μm^2, while the control surface contains 12 000 PLL coils/μm^2. All studies were conducted in pH 7.4 phosphate buffer having an ionic strength of 0.026 M.

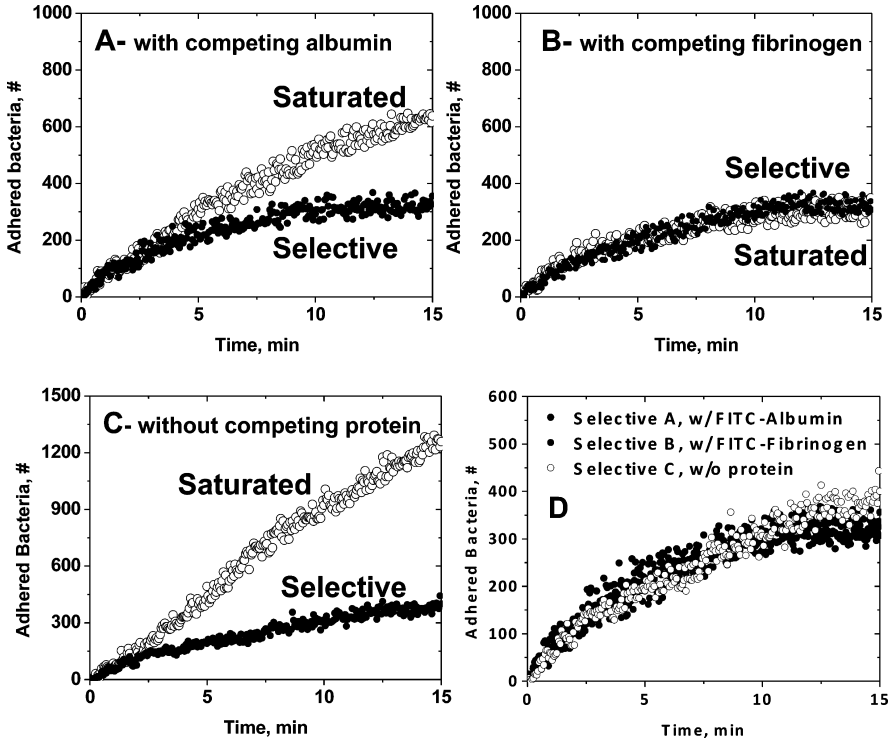

Figure 3. Video microscopy measurements of *S. aureus* capture kinetics from solutions containing FITC-tagged proteins. Bacterial cells are suspended at a concentration of 5×10^5/mL in (A) buffered albumin solution, 2000 ppm; (B) buffered fibrinogen solution, 200 ppm; or (C) phosphate buffer alone. The buffer is pH 7.4 with an ionic strength of 0.026 M. Each plot in panels A−C compares bacterial capture on a selective surface having 3500 PLL/μm^2 to that on a control surface having 12 000 PLL/μm^2. Panel D compares bacterial capture from panels A−C on the selective surfaces, providing a direct quantitative comparison.

Figure 1 reveals that (1) without cationic polymer coils (on the left side of the plot) neither proteins nor bacteria adhere on the PEG brush; (2) the adhesion of each species occurs above a relatively sharp species-dependent adhesion threshold in the surface loading of the cationic polymer coils; and (3) as the PLL coil content of the surface is increased for a series of surfaces along the *x*-axis, bacteria are first seen to adhere to some surfaces and then proteins adhere at higher PLL loadings. Also note that protein coverage on these surfaces is relatively insensitive to first-order to free solution concentration, as is often the case. For instance, the threshold reported for albumin in Figure 1 measured at 100 ppm protein also holds for 2000 ppm albumin. Restating point 3: the adhesion threshold for *S. aureus* occurs to the left, at lower cationic polymer loadings, than the adhesion thresholds for the proteins. Also note that, while this study focuses on fibrinogen and albumin, in a previous work with related surfaces and additional proteins,[28] fibrinogen exhibited the lowest adhesion threshold of the proteins studied. The differences in adhesion thresholds for proteins versus bacteria for these types of surfaces (PEG brushes containing embedded discrete cationic nanoscale features) are significant.

Figure 1, which summarizes the results for individual species in solution, suggests that some surface designs will be adhesive for bacteria but not proteins, when the two are presented in the same solution. In this study, we choose 3500 PLL coils/μm^2 to be the "selective surface" to test this hypothesis in the next phase of study. This composition, which is below the thresholds

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for fibrinogen and albumin adsorption, produces finite capture of *S. aureus*, although not yet at the transport-limited rate. Notably, we could have chosen any surface composition in the range from ~2000 to ~4000 PLL coils/μm^2, and the results are expected to be qualitatively similar but different in terms of the bacteria capture rate. Also note that the exact range of surface compositions where selectivity should occur will be dependent on the architecture of the PEG brush.[28,45] The next phase of this study examines the competitive adsorption of bacteria and proteins from mixed solutions on the selective surface indicated in Figure 1, making comparison to the competitive adsorption on a control surface. The control surface contains 12 000 PLL coils/μm^2 on the right side of Figure 1. Here, both bacteria and proteins individually adsorb at their transport-limited rates.

Capture/Adsorption from Mixed Solutions. In a series of studies, we compared the accumulation of protein and bacteria from mixed protein−bacteria solutions on selective and control surfaces. The study in Figure 2 employed fluorescently tagged albumin and fibrinogen, each mixed individually with 5 × 10^5/mL *S. aureus* and flowed over surfaces in the TIRF instrument. The kinetic TIRF traces in Figure 2 show the time-dependent protein content at the interface, while any bacteria adhesion is not directly evident, because the labels reside on the albumin or fibrinogen.

In Figure 2A, there is no albumin adsorption on the selective surface, whereas, on the control surface, albumin adsorption is substantial and in qualitative agreement with previous reports of albumin adsorption on cationic surfaces.[28] Notably, we saw no adsorption of albumin when the solution concentration was 200 ppm; however, to demonstrate just how high the albumin concentration could be, Figure 2A shows a lack of protein adsorption when the albumin concentration is increased to 2000 ppm. This result with the albumin−*S. aureus* mixture is consistent with Figure 1 in which albumin on its own does not adsorb to a surface containing 3500 PPL coils/μm^2. Figure 1 also anticipates albumin adsorption on the control surface, now in the presence of *S. aureus*. In Figure 2B, with fluorescently labeled fibrinogen, interfacial protein is evident for both the selective and control surfaces. The fibrinogen adsorption on the control surface in the presence of *S. aureus* is consistent with that in Figure 1 for fibrinogen adsorption on its own. However, the interfacial fibrinogen accumulation near the selective surface in Figure 2 seems to be at odds with the expectations from Figure 1, but is a result of fibrinogen−*S. aureus* binding, as revealed below.

Capture/Adsorption from Solutions Containing Protein and Bacteria. While Figure 2 summarizes protein adsorption kinetics from solutions containing both *S. aureus* and protein, Figure 3 tracks bacterial accumulation, measured via video microscopy, for analogous runs. In Figure 3, protein behavior is not evident. One must refer back to Figure 2.

In Figure 3A, it is evident that, in the presence of albumin, *S. aureus* is captured on both selective and control surfaces, as intended. Recalling the lack of albumin adsorption on the selective surface in Figure 2A, it is now evident that the surface is indeed selective for *S. aureus* and rejects albumin when the two are mixed. In Figure 3B, *S. aureus* adsorbs on both selective and control surfaces, as anticipated according to Figure 1. However, because Figure 2B demonstrates that fibrinogen is also present near the selective surface, the actual selectivity of this surface would appear to be in doubt. When fibrinogen is exposed to the selective surface on its own in Figure 1, there is no adsorption; however in the presence of *S. aureus*, fibrinogen accumulates near the selective surface.

Figure 3C reveals, for bacterial capture without competing protein, an additional point: The bacterial capture rates on the selective surface are mostly unaffected by albumin or fibrinogen in solution in Figures 3A or 3B. However, on the control surface, the presence of protein reduces bacterial capture. This is significant because the control surface is an almost-homogeneous cationic surface, typical of antimicrobial cationic surfaces, and demonstrates how, on these more-conventional almost-uniform surfaces, protein fouling reduces bacterial capture. In Figure 3D, bacterial capture on the selective surfaces with and without proteins is directly compared to further emphasize that not only are the test surfaces selective for bacteria, protein fouling also does not alter bacterial adhesion.

Location of Proteins in the Presence of *S. aureus*. Figures 2 and 3 suggest that the single species behavior in Figure 1 is a good predictor for adsorption in albumin/*S. aureus* mixtures but not in fibrinogen/*S. aureus* mixtures. Therefore, we more closely scrutinized the locations of the proteins during bacterial capture, especially the possibility that fibrinogen−*S. aureus* binding brings fibrinogen to the interface in Figure 2B without direct fibrinogen adsorption on the selective engineered surface.

In Figure 4A, fluorescently-tagged albumin (or, in Figure 4B, tagged fibrinogen) is mixed with *S. aureus* and imaged in solution. In the case of albumin, the fluorescence micrograph reveals high fluorescence background signal throughout, and the bacteria are evident only in the bright-field image. The lack of bacterial fluorescence indicates that fluorescent albumin does not bind in solution to *S. aureus*, as expected. In the case of fibrinogen in Figure 4B, fluorescent dots suggest adsorption of fluorescent fibrinogen onto the bacterium in solution, which is consistent with the literature.[36]

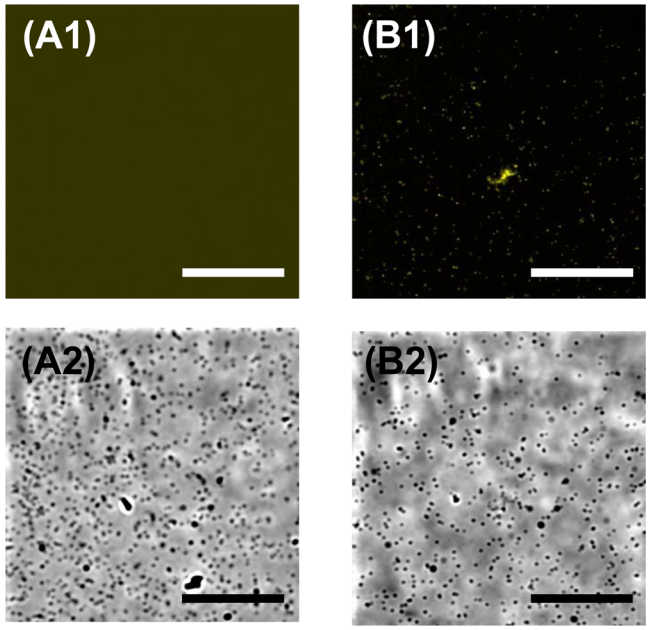

Figure 4. (A1 and B1) Fluorescence and (A2 and B2) bright-field micrographs of *S. aureus* mixed with FITC-albumin (panels A1 and A2) and FITC-fibrinogen (panels B1 and B2). Scale bars = 50 μm.

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The micrographs in Figure 4 image *solutions* containing fluorescently labeled protein and bacteria. Figure 5 presents

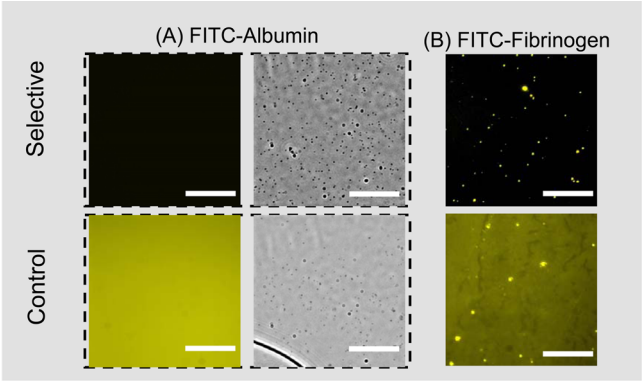

Figure 5. Fluorescent and bright-field images of selective and control surfaces exposed to (A) FITC-albumin or (B) FITC-fibrinogen solutions each containing 5×10^5/mL *S. aureus*. Scale bars = 50 μm.

micrographs of selective and control *surfaces* exposed to solutions that contain bacteria mixed with fluorescently labeled protein. In these studies, surfaces are exposed to flowing test solutions, and then rinsed with flowing buffer in the flow chamber before transfer to the fluorescence microscope.

In Figure 5A, for surfaces exposed to fluorescein-albumin (2000 ppm) and *S. aureus* (5×10^5 cells/mL), the lack of fluorescence on the selective surface follows the predictions of Figure 1 for a lack of albumin adsorption. This observation concurs with the quantitative fluorescent trace in Figure 2A. Also in Figure 5A, the highly fluorescent control surface confirms expectations from Figure 1 for substantial albumin adsorption on the control surface. In the corresponding bright-field micrographs of Figure 5A, bacteria adhere to both selective and control surfaces, as designed. Notably, the bacteria in Figure 5A do not fluoresce, because albumin does not associate with *S. aureus*, as demonstrated in Figure 4. To summarize, in the case of albumin−*S. aureus* mixtures, albumin and bacteria adsorb independently, such that, from a mixture, their adhesion to selective or control surfaces follows the predictions of Figure 1, based on single species adsorption.

Different behavior is seen in Figure 5B for surfaces exposed to fluorescein-fibrinogen/*S. aureus* mixtures. Here, bacteria capture, which occurs by design on both selective and control surfaces, is evident in the fluorescence micrographs because fluorescein−fibrinogen binds directly to *S. aureus*. In the micrographs of Figure 5B, the parts of the surface that do not contain bacteria are dark or fluorescent, on the selective and control surfaces, respectively, which is consistent with fibrinogen adsorption in Figure 1. Figure 5B establishes that (i) the interfacial fluorescence and apparent lack of selectivity in Figure 2B are a result of fibrinogen binding to *S. aureus* and (ii) the engineered selective surface actually is not fouled by fibrinogen. Even when fibrinogen binds to *S. aureus*, we can claim that the surface is selective, because the fibrinogen binds to the bacteria and resides at the interface but not directly on the surface.

DISCUSSION

This program developed a strategy for the design of surfaces that selectively capture bacteria from protein-containing solutions. This is a general challenge because proteins typically adsorb to surfaces via the same nonspecific (van der Waals, hydrophobic, electrostatic) interaction that also drive bacterial adhesion. By employing surfaces that are generally repulsive (here, via steric interactions) but localize attractive interactions (here electrostatic), and by making the discrete electrostatic interactions extremely weak, we have overcome this challenge. The individual electrostatic patches attract proteins or bacteria too weakly for a single patch to retain either proteins or bacteria. The need for multiple patches to capture bacteria or proteins is manifest in the adhesion thresholds, distinct for each protein or bacteria species, in Figure 1. (The concept of surface binding multivalency and its specific role in the capture of particles, proteins, and bacteria was previously developed separately for a broad class of surfaces,[46,47] and specifically for the PLL−PEG brush surfaces like those here.[27−29]) These thresholds are dependent, based on the numbers of patches needed to capture an object, not only on the weak binding energy between the object and the patches but also on the steric repulsion from the brush. The total steric repulsion is dependent on both the local object shape and on the architecture of the brush. Indeed, qualitatively similar adhesion thresholds had been previously reported for a slightly different brush,[29] and, furthermore, a series of thresholds for different proteins had also been reported.[28]

It is generally observed, in Figure 1, that the adhesion thresholds for proteins lie to the right of those for bacteria under these flow and ionic conditions (perhaps as a result of the smaller protein size). Thus, these patchy surfaces exhibit a tendency to capture bacteria instead of proteins. A strategy, then, for the selective capture of bacteria in the presence of proteins is to design collecting surfaces with patch loadings exceeding those of the bacterial thresholds but below the thresholds for protein adsorption.

The results in this study confirmed that this strategy for the design of *S. aureus*-targeting surfaces does, indeed, work. A definition of selectivity, typically applied to competing cell types or separately to competing molecules, is not meaningful for competition between cells and molecules, at least to any precision. However, we do report that, for the case of bacterial capture in the presence of proteins such as albumin, which do not substantially bind to the bacterium, the approach of surface design based on adhesion thresholds provide excellent selective capture of bacteria: No albumin was found on the surface in the competitive adsorption experimental within detectable limits of 0.03 mg/m^2 for this particular experimental configuration. Furthermore, the amount of bacteria captured in competition the selective surface was identical, within experimental error, to that of *S. aureus* binding without protein in solution.

We also demonstrated that the surface design strategy that was guided by the adhesion thresholds works very well in the case where the dissolved protein associated specifically with the targeted bacteria. Of course, when the *S. aureus* and fibrinogen were mixed in solution, capture of fibrinogen on the collector did not unbind the fibrinogen from the bacteria; therefore, fibrinogen was present at the interface, but not on the collector itself. Furthermore, in the specific case of fibrinogen and *S. aureus*, fibrinogen on the bacterial surface did not prevent the bacterial capture. This favorable result may have occurred because the attractive patches on the collector operate through long-range electrostatic attractions rather than a lock-key recognition.

One important observation regarding patchy brush surfaces engineered between the adhesion thresholds of competing

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species: This approach eliminates competition for the surface, because the protein simply does not adsorb to the surface below its threshold. By comparison with the uniformly cationic collectors, adsorbing proteins reduce the bacterial binding through competition. Therefore, not only do the patchy surfaces exhibit selective capture of bacteria, the yield of bacterial capture is greater than on the control surface, because proteins do not obstruct bacterial binding on the selective surface.

Finally, this surface design strategy, based on adhesion thresholds, is likely to be broadly generalizable, to the extent that bacteria of interest adhere at thresholds below those of proteins. We focused our efforts here on the study of *S. aureus*, because the effort needed to study bacterial capture with this level of quantitative depth is limited by our resources. However, we have not tried other bacteria where this approach as failed. Also note that fibrinogen was chosen for the current project because its threshold is closest to that *S. aureus*, potentially giving worst-case protein behavior, complicated by its direct binding to *S. aureus*. Therefore, the strategy of choosing surfaces between adhesion thresholds for bacterial capture from protein solutions is likely to work as a general strategy. We also expect modest variations in ionic strength to be nonlimiting: We demonstrated a shifting in thresholds with ionic strength (in the same direction) for both *S. aureus*[29,31] and fibrinogen,[45] so that the findings for selectivity reported here would be expected to hold qualitatively with variations in salt. Finally, we note that single-species protein and bacteria thresholds reported, such as those reported, for instance, in Figure 1, are typically independent of the concentration of the analyte species. However, more dilute solutions may take longer, for reasons of diffusion, to accumulate on an adhesive surface. Without a change in adsorption mechanism at elevated concentrations, we expect that adsorption/capture thresholds will remain as reported and this will translate to robust surface selectivity.

CONCLUSIONS

We have demonstrated that bacteria can be selectively captured from protein solutions on surfaces containing nonmolecularly specific surface chemistry. By sparsely embedding cationic polymer coils at the base of a sterically repulsive polymer brush, it becomes possible to identify collector compositions with sufficient adhesive functionality to capture bacteria but not enough to capture proteins. Bacteria then adhere from protein solutions without the protein adsorption that fouls the surface and reduces bacterial adhesion on more classically adhesive surfaces. With the thresholds sufficiently spaced, as was the case for fibrinogen, albumin, and *S. aureus*, it is possible to achieve extremely high selectivity. The bacterial capture approached the transport limit, while no protein could be detected on the surface. The direct association of protein with the bacteria surface did not impede bacterial capture.

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Notes

The authors declare no competing financial interest.

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