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Time-of-flight secondary ion mass spectrometry, fluorescence microscopy and scanning electron microscopy: Combined tools for monitoring the process of patterning and layer-by-layer assembly of synthetic and biological materials

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ABSTRACT

We have employed time-of-flight secondary ion mass spectrometry (TOF SIMS), fluorescence microscopy and scanning electron microscopy (SEM) to monitor the immobilization of biotinylated shell-crosslinked nanoparticles (SCKs) on biotin/streptavidin-functionalized, UV-photopatterned self-assembled monolayers (SAMs). TOF SIMS and fluorescence microscopy images showed that the streptavidin was immobilized primarily in the biotin-functionalized SAM areas. Biotinylated SCKs underwent both streptavidin–biotin recognition and electrostatic interactions to the underlying substrate. Upon adsorption, the biotinylated SCKs deformed significantly; their cross-sectional diameter increased by ~36% from 65 ± 7 nm to 90 ± 2 nm. Using the SCK contact area, we estimate that one SCK was bound between one and five streptavidin proteins. These data suggest that functionalized SCKs can be employed as biomolecule mimics to investigate the factors that control biomolecule adsorption on functionalized surfaces.

1. Introduction

The immobilization of biomolecules on patterned surfaces has many important technological applications, including in biosensing [1,2], diagnostics [3] and drug discovery [4,5]. A variety of substrates has been used to immobilize biomolecules, including self-assembled monolayers (SAMs), polymer brushes and hydrogels [6–19].

Biotin–streptavidin constructs have been widely employed as biosensor platforms [1]. The broad applicability is due, in large part, to the availability of streptavidin, the simple chemical modification of biotin, and the high binding affinity ($k \sim 10^{15}$ M^{-1}) between the two [20,21]. Streptavidin has four equivalent binding sites (two on one side and two on the on the opposite side). These sites can be used to link streptavidin nearly irreversibly with up to four biotinylated molecules. There is often little impact on the biological activity of these secondary biotinylated molecules.

The use of SAM substrates for biomolecule immobilization is attractive for several reasons. SAMs have highly organized, well-defined structures with a uniform density of terminal groups, which allows facile control over their surface chemistry and reactivity [9,22–25]. For example, oligo(ethylene glycol) (OEG)-functionalized SAMs have been shown to resist the non-specific adsorption of enzymes and proteins effectively [26–31]. Additionally, SAMs can be patterned using a variety of methods, including microcontact printing [32–35], nanoimprinting [36], energetic beam lithography [37,38], scanning probe-based nanolithographies [4,39,40] and UV photopatterning [41–45].

There have been many studies of the interaction of streptavidin (SA) with biotinylated SAMs [25,46–52]. There are many factors that affect the kinetics of the biotinylated SAM–streptavidin interaction, including the surface coverage of the biotin functional groups. At low surface coverage, the SA-biotin surface is primarily composed of singly bound SA [49,51]. As the biotin surface coverage increases to ~30%, SA binds to two biotins [49,51]. However, at higher coverages (above ~40%), SA only binds to one biotin molecule [49]. These studies showed that the optimal streptavidin surface coverage is ~30% [25,49,51] and, further, that the optimal SA surface coverage is dependent on the chemical identity of the second SAM in the mixed monolayer [25].

There have been few studies of the adsorption of a second biotinylated-molecule layer on these SA-biotin SAM constructs. Cui

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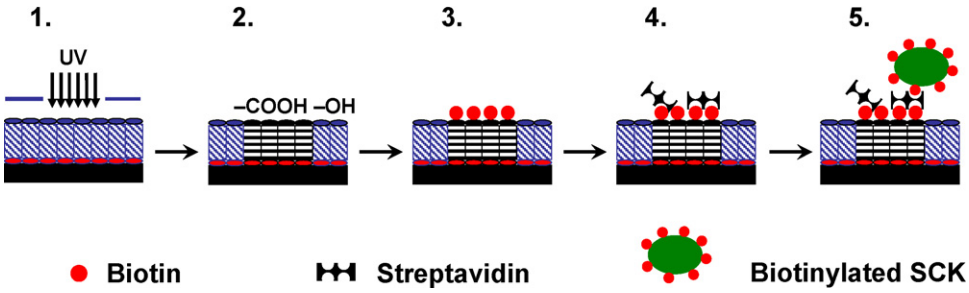

Fig. 1. Schematic illustration of the five stages of the selective SCK immobilization process: (1) photopatterning of SAM #1 (EG3OH); (2) formation of SAM #2 (EG6COOH) by displacement of photoreacted molecules; (3) conjugation of amine-functionalized biotin to EG6COOH SAM; (4) attachment of streptavidin (SA-488) on top of biotin layer; and (5) immobilization of SCK on top of the streptavidin layer.

et al. [53] demonstrated that multilayer films of avidin and biotin-labeled antibodies could be employed as immunosensors. However, they also observed that surface-immobilized streptavidin bound less than one biotinylated antibody on average ($S \sim 0.4$, where S is the stoichiometry of the biotin–avidin interaction). These results demonstrate that further work is required to understand the binding of biotinylated molecules to SA-functionalized surfaces.

Our interest in the use of streptavidin as a digestable protein layer from which synthetic nanoparticle rafts could be assembled, stabilized and later lifted led us to investigate, first, the ability to assemble synthetic polymer nanoparticles upon a patterned surface of streptavidin. Therefore, in this paper, we have constructed a bio-synthetic hybrid multi-layered structure by assembling biotinylated shell-crosslinked nanoparticles (SCKs) [54–56] onto a patterned streptavidin/biotin SAM surface. SCKs have many similar properties to biological macromolecules, such as proteins [57]. They are polymeric nanostructured materials with a hydrophobic core and a hydrophilic shell layer, which is often charged and highly hydrated in an aqueous environment. At each step of the assembly the chemistry and structure of the construct was monitored using a combination of time-of-flight secondary ion mass spectrometry (TOF SIMS), fluorescence microscopy and secondary electron microscopy. To aid in the discussion of the results, a schematic diagram of the overall multi-layer surface assembly processes is shown in Fig. 1.

2. Experimental

2.1. Materials

All materials and reagents were used as received. Hydroxyl- and acid-terminated alkanethiols with ethylene glycol (EG) segments, $HS(CH_2)_{11}(OCH_2CH_2)_3OH$ (EG3OH) and $HS(CH_2)_{11}(OCH_2CH_2)_6OCH_2COOH$ (EG6COOH) were purchased from Prochimia. Absolute ethanol (99.5%), *N*-hydroxysuccinimide (NHS), 1-(3′-dimethylaminopropyl)-3-ethylcarbodiimide methiodide (EDCI), (+)-Biotin (99%), Tween 20 and HEPES (99.5%) were purchased from Aldrich. Alexa Fluor 488 conjugated streptavidin (SA-488) was obtained from Molecular Probes, Inc. Si ⟨1 1 1⟩ wafers were obtained from Addison Technologies, Inc. Chromium and gold were purchased from Goodfellow and Alfa Aesar and were of ≥99.99% purity.

Biotinylated SCKs were prepared from their block copolymer precursor, poly(acrylic acid)-*block*-polystyrene (PAA-*b*-PS), following a three-step procedure: micellization, shell crosslinking, and biotinylation [58].

2.1.1. Micellization

Block copolymer PAA_{70}-*b*-PS_{560} (1.000 g, 0.0158 mmol, $M_n^{SEC} = 63200$ g/mol, $M_w/M_n = 1.10$) was dissolved in THF (500 mL) to form a solution with a concentration of ~2 mg/g. After allowing the solution to stir overnight at room temperature, PBS buffer (500 mL, 5 mM sodium phosphate, 5 mM sodium chloride, pH 7.4) was added at a rate of 15 mL/h *via* syring pump with continuous stirring. After buffer addition was complete, the micelle solution was added slowly into PBS buffer (1000 mL, 5 mM sodium phosphate, 5 mM sodium chloride, pH 7.4), and allowed to stir overnight in order to further stabilize the micelle. The remaining THF was removed by membrane tubing dialysis (Spectra/Por membrane tubing with MWCO 6000–8000 Da) against nanopure water (18 MΩ cm) to afford micelles (3230 g, 0.309 mg/g). DLS (dynamic light scattering) (volume-average): $D_h = 86 \pm 2$ nm.

2.1.2. Shell crosslinking of micelles

A solution of micelles (500 mL, 0.309 mg/g), prepared from the block copolymer precursor, was incubated with 2,2′-(ethylenedioxy)diethylamine (13.3 mg, 89.4 μmol, 0.5 molar equiv., amino to carboxylic acid groups) for >2 h. EDCI (53.2 mg, 0.179 mmol) was diluted with H_2O (2 mL) and added to the solution of dropwise. The reaction mixture was allowed to stir for ~14 h, followed by dialysis (Spectra/Por membrane tubing with MWCO 6000–8000 Da) against deionized water for 2 days and against nanopure water for another 2 days to obtain an aqueous solution of SCKs (498 g solution, 0.310 mg/g). DLS (volume-average): $D_h = 65 \pm 7$ nm. TEM (transmission electron microscopy): $D_{av} = 62 \pm 8$ nm.

2.1.3. Biotinylation of SCKs

A biotinylated amine, *N*-biotinyl-3,6-dioxaoctane-1,9-diamine, was synthesized according to the procedure of Sigal et al. [59]. SCKs were incubated with the biotinylated amine for >2 h with stirring. EDCI was added dropwise as a freshly prepared aqueous solution. The solution mixture was allowed to stir for ~14 h, followed by dialysis (Spectra/Por membrane tubing with MWCO 6000–8000 Da) against nanopure water for >4 days to obtain aq. solution of biotinylated SCKs.

2.2. UV-photopatterned SAMs

2.2.1. SAM Preparation

The preparation and characterization of the types of SAMs used in this study have been described in detail previously [22,24,22,60,61]. Briefly, the Si wafers were etched using Piranha etch (H_2SO_4:H_2O_2 = 7:3, v/v) for 1 min, rinsed with copious amounts of distilled water and ethanol, and then dried using nitrogen gas. Cr (~50 Å,) and Au (~1000 Å) were then thermally deposited sequentially onto Si wafers. Self-assembled monolayers were prepared by immersing the Au substrate in a 2 mM EG3OH ethanolic solution for 24 h at ambient temperature (21 ± 1 °C).