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ARTICLE *in* LANGMUIR · MAY 2012

Impact Factor: 4.46 · DOI: 10.1021/la301441t · Source: PubMed

Langmuir

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Dopamine-Assisted Rapid Fabrication of Nanoscale Protein Arrays by Colloidal Lithography

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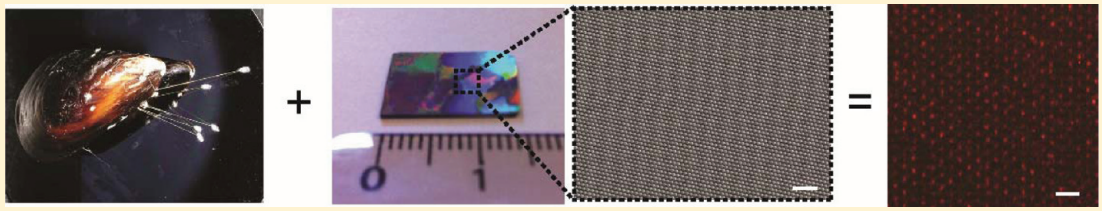
Supporting Information



ABSTRACT: The development of cost-effective methodologies for the precise nanometer-scale positioning of biomolecules permits the low-cost production of various biofunctional devices for a range of biomedical and nanotechnological applications. By combining colloidal lithography and the mussel-inspired multifunctional polydopamine coating, we present a novel parallel benchtop method that allows rapid nanoscale patterning of proteins without the need for electrically powered equipment in the fabrication process. The PDA-immobilized binary nanopattern consisting of BSA surrounded by PLL-*g*-PEG is fabricated over a large area, and the integrity of the pattern is confirmed using AFM and FM.

INTRODUCTION

The engineering of new methodologies for the precise surface positioning of specific proteins is critically important in biological applications, particularly for biosensing, drug screening, medical diagnostics, and conducting fundamental cell studies.[1] The recent development in the area of protein patterning has been focused on the ability to position proteins accurately in the nanometer range with controlled orientation and density in a low-cost, high-throughput manner. By taking these criteria into account, numerous approaches have been pioneered in recent years, including SPM-based pen lithography[2] as well as nanoimprint lithography[3] and nanocontact printing[4] that permits the nanoscale parallel patterning of biological entities. However, these techniques together with other existing patterning methodologies, as discussed in several reviews,[1a,b,d,S] generally require expensive specialized equipment and clean room facilities. The alternative bottom-up-only methodologies of block copolymer micelle lithography[5,6] and colloidal lithography[7] can cost-effectively produce a range of chemical and biological patterns with various sizes and shapes in a parallel manner.

To generate protein patterns, the proteins should be immobilized via the covalent- or affinity-based coupling route because the direct physical adsorption of proteins onto substrate surfaces can lead to as much as 90% in the denaturation of adsorbed proteins, leading to a loss of activity.[1a] To control and retain protein orientation and activity, a common approach is to pattern chemical moieties consisting of protein-resisting and protein-coupling regions. Colloidal lithography has been extensively used as a cost-effective template to generate these regions on the micro/nanoscale (e.g., (1) via the use of PVD first to fabricate different material patterns, followed by the self-assembly of material-specific chemically functional monolayers,[8] (2) via the use of plasma polymerization;[9] or (3) both[10]). Although these approaches are relatively straightforward, expensive deposition equipment and multiple processing steps are required to achieve a covalently coupled biomolecular surface pattern.

Inspired by nature, the discovery and development of a multifunctional polydopamine (PDA) coating from mussel foot protein have led to intense research into the use of PDA in a variety of applications because of its physical (virtually applicable to any surface) and chemical (via the reactive catechol/quinone groups present on the PDA surface) versatility.[11] However, the use of PDA in the area of biological surface patterning has so far been limited, with only a few surface-patterning techniques such as microcontact printing[12] and microfluidics[13] having been used to pattern biological cells using PDA as a cell adhesion layer surrounded by a cell-resistant region. The attachment of biomolecules onto PDA surfaces with the retention of biological activity and control over its orientation via pH has been demonstrated on a flat substrate,[14] thus PDA can be an effective material candidate for patterning and tethering biological molecules onto surfaces.

EXPERIMENTAL SECTION

Experimental details are provided in the Supporting Information.

Received: April 10, 2012
Revised: May 23, 2012
Published: May 28, 2012

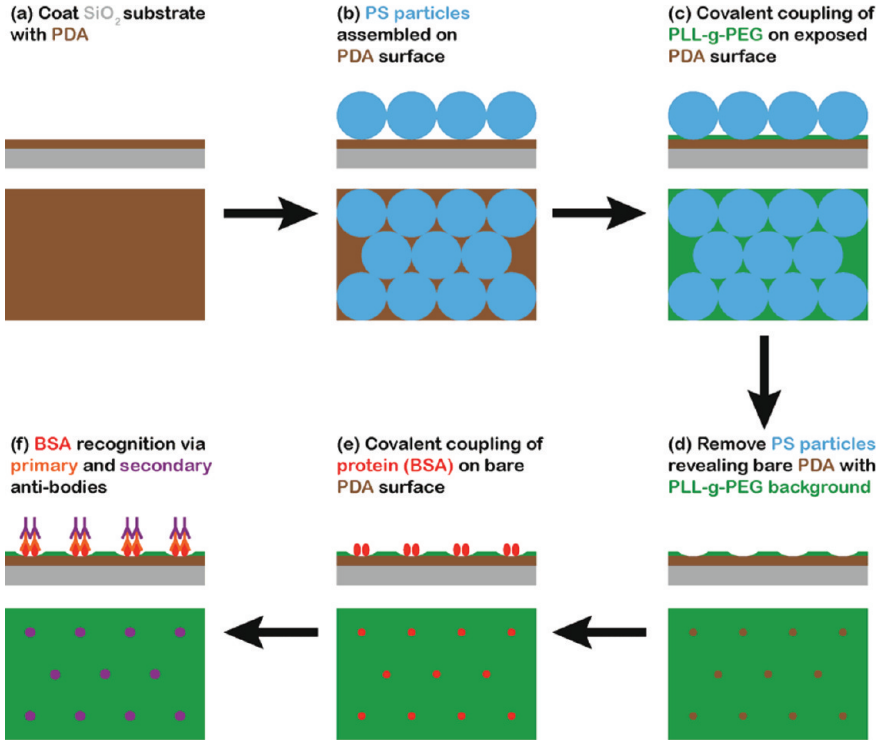


Figure 1. Dopamine-assisted colloidal lithography process, showing the side view (top) and top view (bottom, not to scale). A substrate (SiO_2) is first coated with polydopamine (PDA, a), and the polystyrene (PS) particles are self-assembled on the PDA-coated surface (b). The entire surface is then immersed in PLL-*g*-PEG to make the background nonfouling (c). Removing PS particles reveals a binary pattern consisting of a bare PDA periodical array surrounded by PLL-*g*-PEG (d). The surface is immersed in protein solution, where the catechol/quinone group reacts with a free amine group to immobilize proteins onto the PDA surface (e). The recognition of the attached protein by primary/secondary antibodies (f).

■ RESULTS AND DISCUSSION

We demonstrate herein for the first time, a straightforward benchtop method for creating a large-area nanoscale array of proteins surrounded by a nonfouling region by using a combination of colloidal lithography and PDA. The presented technique is extremely simple and cheap and requires no electrically powered equipment for fabrication. Figure 1 represents a schematic of the overall patterning process. A substrate is coated with PDA first, then PS particles are self-assembled into an HCP structure on the PDA-coated substrate by lifting the PS particles assembled on the air−water interface, as described previously by our group[10] over a large area (typically ∼1 cm × 1 cm, photographic and scanning electron micrographic images of the assembled particles are given in Figure S1a,b respectively). We have employed SiO_2 as a substrate for this study, but because of the multimaterial coating capability of PDA,[11] virtually any substrate can be employed. The PDA substrate assembled with PS is immersed in poly-L-lysine-grafted poly(ethylene glycol) (PLL-*g*-PEG). As shown in previous studies,[11,14,15] it is possible to covalently conjugate the free amine moieties from PLL with the catechol/quinone groups present at the PDA surface via Schiff base/Michael addition reactions. Interestingly, the PS particles were held in position on the surface by PDA during the incubation in PLL-*g*-PEG solution. This may be due to the "sticky" nature of PDA, as previously observed by another group.[12] Usually, mild heating of the self-assembled PS templates is required upon immersion in solution because the particles could easily lift off from the substrate.[10] The region where the PS particles are in contact with the PDA is inaccessible to the PLL-*g*-PEG solution, thus this area of the PDA surface remains unreacted. After incubation in PLL-*g*-PEG, the surface is rinsed with Milli-Q water and the PS particles are lifted off using clean-room tape, revealing a binary pattern consisting of the bare PDA region surrounded by the PLL-*g*-PEG-coated region. The surfaces are immersed in a solution of protein and directed to the bare PDA region. Finally, the immobilized proteins can be site-specifically recognized by primary and secondary antibodies.

In the presented technique, the aggregates of PDA microparticles that often form during the polymerization of dopamine should be reduced in size and number and if possible eradicated because (1) the presence of large PDA aggregates may affect the self-assembly of PS particles and (2) firm contact between a particle and PDA is required to generate bare PDA regions that are inaccessible to the PLL-*g*-PEG solution. The originally reported PDA concentration of 2 mg/mL[11] produced large micrometer-scale aggregates of PDA on the surface, thus it is not suitable for the subsequent PS particle assembly. By investigating the effects of concentration and incubation time of PDA, the concentration of 1 mg/mL for 60 min is found to be sufficient to obtain an ultraflat PDA film on SiO_2 with the minimal size and number of aggregates on the surface while retaining a continuous PDA film. A representative atomic force microscopy (AFM) image of the PDA coatings is given in Figure S2.

Prior to conducting surface patterning, XPS was employed on the flat substrates to confirm the following: (1) a PDA coating on a SiO_2 substrate; (2) the attachment of PLL-*g*-PEG to PDA (PDA-PLL-*g*-PEG); and (3) the attachment and repellency of model protein bovine serum albumin (BSA) on PDA and PDA-PLL-*g*-PEG, respectively (Figure 2, Figure S3,

dx.doi.org/10.1021/la301441t | *Langmuir* 2012, 28, 8594−8599

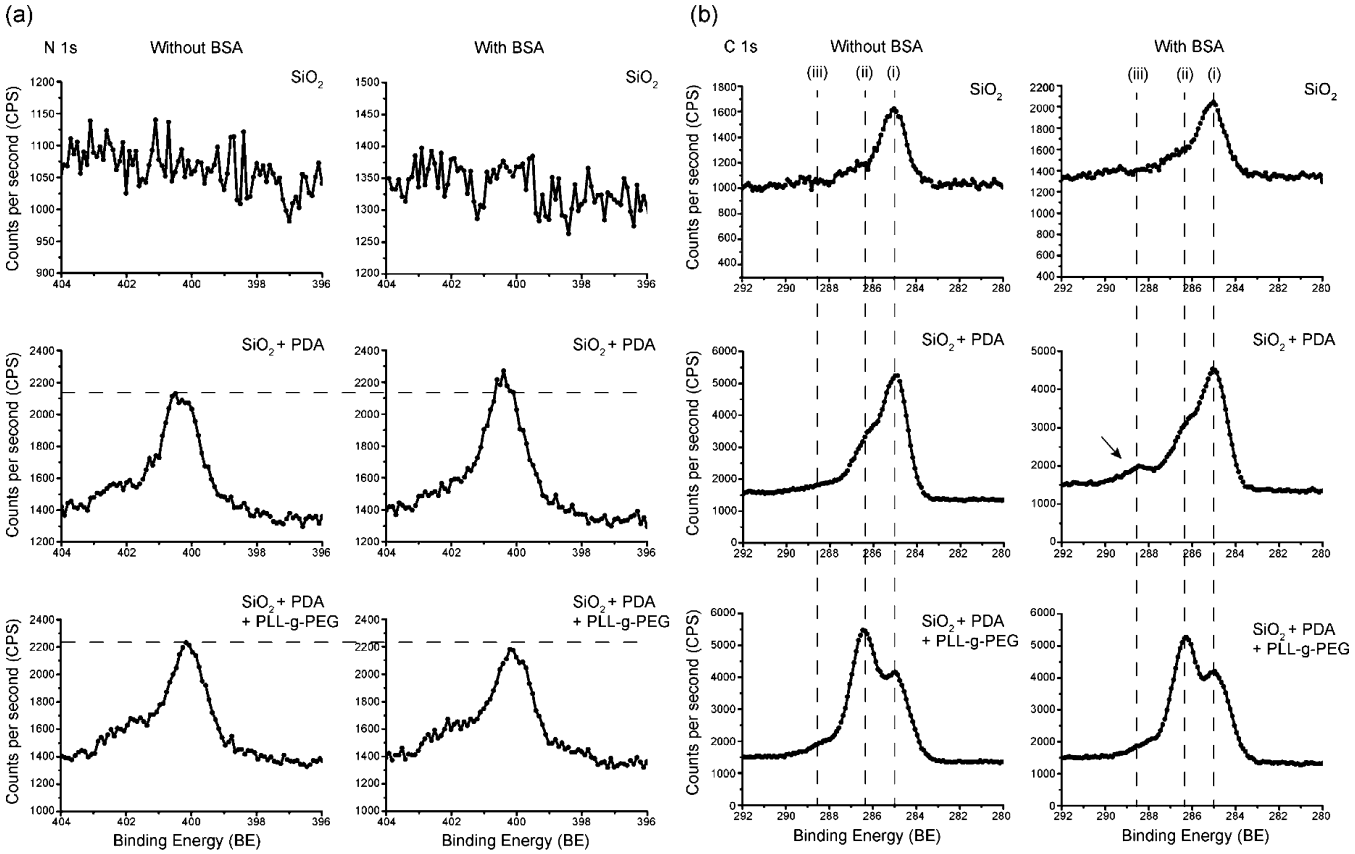


Figure 2. High-resolution XPS N 1s (a) and C 1s (b) spectra of bare SiO_2, PDA-coated SiO_2 and PDA, and PLL-*g*-PEG-coated SiO_2 with and without exposure to BSA. The C 1s is highlighted mainly by three regions: the *C*−C/*C*−H region at BE = 285.0 eV (i), the *C*−N/*C*−O region at BE ≈ 286.5 eV (ii), and the *C*(═O)−N/*C*(═O)−O region at BE ≈ 288.5 eV (iii). An arrow in C 1s and the slight increase in the maximum N 1s intensity at BE ≈ 400.0 eV indicate the adsorption of BSA on PDA.

and Table S1). The successful deposition of PDA and the subsequent conjugation of PLL-*g*-PEG onto PDA are evident from the emergence of a peak at BE ≈ 400.0 eV in the N 1s region, indicative of PDA (Figure 2a), and the increase in the C−O component at BE ≈ 286.5 eV in the C 1s region, indicative of PEG (Figure 2bii), respectively. Upon incubation of the surfaces in BSA, negligible differences in N 1s surface elemental concentrations were observed in the survey scan (Figure S3). The high-resolution scans further revealed that the bare SiO_2 and PDA-PLL-*g*-PEG surfaces have resisted BSA adsorption but the immobilization of BSA on the PDA surface was successful, as evident from the increase in the C(═O)−N region at BE ≈ 288.5 eV (Figure 2iii, indicated by an arrow) and the slight increase in the N 1s intensity observed for the PDA surface. The lack of the physical adsorption of BSA onto bare SiO_2 is most likely due to the small concentration of BSA employed in the incubation (10 μg/mL) and the electrostatic repulsion between the SiO_2 surface and the BSA[16] because the isoelectric points (pI's) of the SiO_2 surface and BSA are below the incubated pH of 7.4.[17] By monitoring the change in the elemental ratios upon BSA incubation for all surfaces (Table S1), a decrease in the C/Si ratio of 23% was observed from the PDA surface incubated in BSA. This change is beyond the expected XPS typical experimental error of ±10%,[18] indicating a potential heterogeneity of the PDA film. This may be caused by (1) the presence of localized PDA aggregates contributing to the variation in the Si signal and/or (2) the geometrical variation in the film thickness of the PDA film, where the film thickness (excluding the aggregates) is expected to be within the analysis depth of XPS. The increase in the N/C ratio change of 23% upon incubation of the PDA surface in BSA is in accord with the high-resolution C 1s and N 1s spectral change observed, confirming that BSA can be immobilized only on the bare PDA regions.

For surface protein patterning, we employed PS particles of diameter d = 1770 nm (PS 1770) for the fabrication of PLL-*g*-PEG and the PDA binary pattern and imaging using AFM (Figure 3a). To demonstrate that particles of different sizes can be employed in the fabrication, PS particles with d = 722 nm (PS 722) were also used to fabricate the binary pattern (Figure S4). The average sizes of the unreacted bare PDA patches are found to be 363 ± 24 and 234 ± 19 nm for PS 1770 and PS 722, respectively. For both sizes of PS, the regions of bare PDA are clearly visible, as envisaged by the corresponding center-to-center distance between PDA regions separated by the diameter of the PS particles (i.e., approximately 1770 and 722 nm). We employed the binary pattern fabricated from PS 1770 to demonstrate the attachment of BSA and the subsequent recognition by anti-BSA on bare PDA regions using AFM. The successful immobilization of BSA and the subsequent recognition by anti-BSA on the bare PDA regions were confirmed by the increase in the height of the regions upon sequential immersion in BSA followed by anti-BSA (Figure 3b, right, indicated by black arrows). We also imaged the surface after the incubation in BSA using AFM, and we observed the disappearance of the indentation of the bare PDA

dx.doi.org/10.1021/la301441t | *Langmuir* 2012, 28, 8594−8599

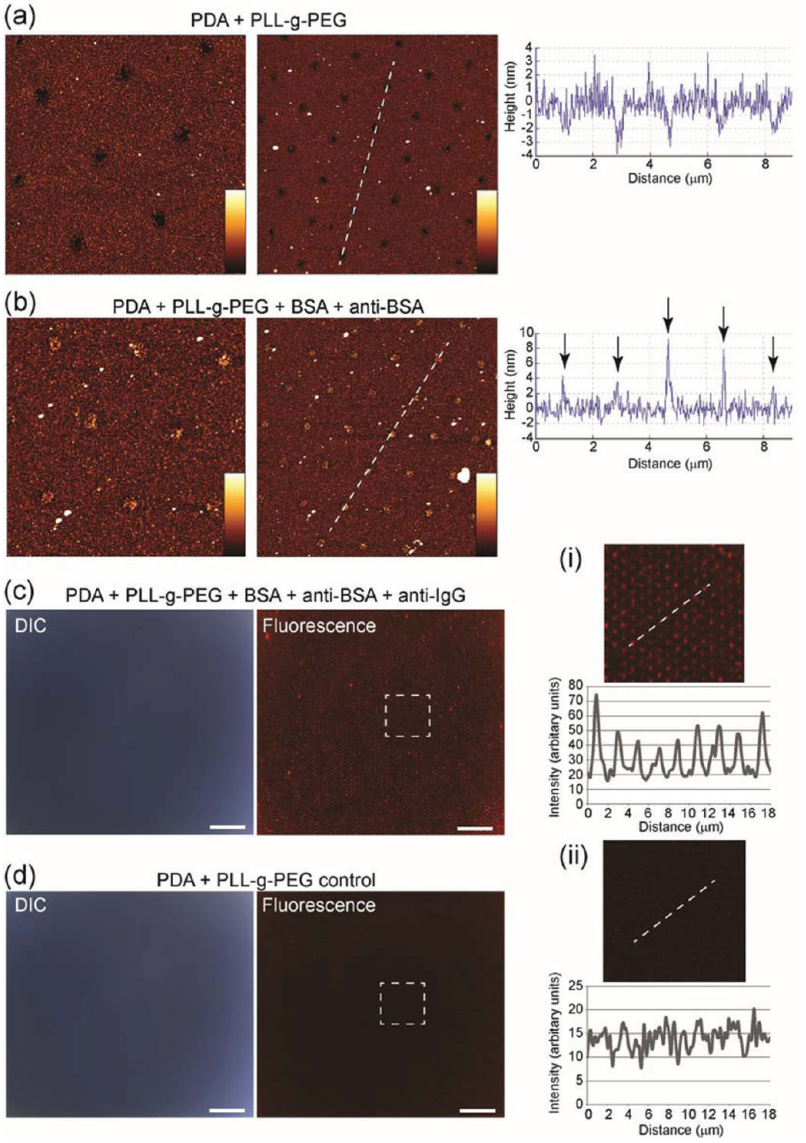


Figure 3. AFM images (5 μm × 5 μm (left) and 10 μm × 10 μm (middle)) of the binary periodical pattern of the bare PDA region surrounded by the PLL-*g*-PEG region fabricated from PS 1770 before (a) and after (b) sequential incubation in BSA and anti-BSA. The scale bar is 0 to 10 nm. The cross-sectional profiles (indicated by the white dashed line on each 10 μm × 10 μm image) and change in height (right) measured by AFM confirm the successful coupling of BSA and the subsequent recognition by anti-BSA. The FM image (middle) highlights the successful recognition of anti-BSA by the rhodamine-labeled anti-IgG secondary antibody (c). To check for autofluorescence, the FM image of the PDA + PLL-*g*-PEG surface was also captured under the same exposure conditions (d). Corresponding DIC images are also given (left). The cross-sectional profile of the enlarged area (2×, highlighted by white dashed boxes) indicates the periodic distribution of the higher-intensity spots separated by the diameter of the particles (∼1770 nm, i) but not on the control (ii), indicating that the fluorescence signals were the results of secondary antibody recognition, not autofluorescence. The scale bar is 20 μm.

regions that were present prior to the incubation (not shown). Finally, to highlight the applicability of the method over a large area, further recognition of the patterned anti-BSA by the secondary antibody (rhodamine-labeled antirabbit IgG secondary antibody) was confirmed using fluorescence microscopy (FM, Figure 3c). The cross-sectional profiling of the enlarged area (highlighted by a white dashed box, 2× magnification of the original image, Figure 3i) revealed that the secondary antibodies have successfully attached to the anti-BSA, with the highest fluorescence intensity regions separated by particle diameters of ∼1770 nm. The control surface consisting of PLL-*g*-PEG with bare PDA was also imaged using FM under the same exposure conditions to confirm that the secondary antibody pattern is not a result of the autofluorescence from the PDA/PLL-*g*-PEG pattern (Figure 3ii).

The exact nature of the coupling between PDA and BSA/PLL-*g*-PEG is difficult to identify and quantify from this study alone. It is likely that the binding between PDA and BSA/PLL-*g*-PEG is a result of both a covalent coupling reaction and physical adsorption. Gaining information on the presence and extent of covalent bonds formed between catechol/quinone groups of PDA and biomolecules is possible via the use of vibrational spectroscopy.[19] It is also worth noting that the Schiff base/Michaels reaction via amine groups may not always be a useful conjugation route for some applications such as biosensors, where, for example, the immobilization of antibodies could occur via the terminal amine moieties on the antigen

dx.doi.org/10.1021/la301441t | *Langmuir* 2012, 28, 8594−8599

binding sites. In this case, covalent antibody immobilization should be ideally carried out via the carboxylic acid group, and because this group is not expected to react to form a covalent bond with the catechol/quinone groups, additional step such as the use of a heterobifunctional linker will be required.

CONCLUSIONS

We have presented a novel and extremely simple method that permits the rapid fabrication of nanoscale protein arrays using bottom-up colloidal lithography combined with a mussel-inspired PDA coating. The use of the PDA coating eradicates the requirement of a specific material surface for the subsequent SAM assembly that is required in a traditional colloidal chemical/biological patterning approach and permits the use of virtually any substrate material for the fabrication. Because of the multichemical functionality provided by the presence of catechol/quinone moieties, the presented technique also allows the patterning of proteins and a protein-repellant region over a large area in a single step. In this study, we have successfully fabricated and confirmed the integrity of the surface pattern consisting of the protein-coupling region of bare PDA and the protein-repellant region of PLL-*g*-PEG and the subsequent coupling of BSA and its recognition by primary and secondary antibodies. The presented method provides a direct economical route to the nanoscale covalent patterning of biological molecules, and we believe that this strategy could be applied to produce functional nanopatterned surfaces inexpensively for a wide range of biomedical applications. Future work is directed toward the characterization of covalently coupled biomolecules on PDA via vibrational spectroscopy, demonstrating the applicability of the method toward a range of cost-effective substrates, and the investigation of the stability of the fabricated biomolecular arrays over an extended period.

ASSOCIATED CONTENT

Supporting Information

Experimental details. Photographic and SEM images of self-assembled PS 1770 particles on PDA-coated SiO_2. AFM images of bare and PDA-coated SiO_2. Relative surface atomic percentages of SiO_2, PDA, and PLL-*g*-PEG and the percentage change in the elemental ratio with and without BSA determined from XPS survey scans. AFM images of the PDA/PLL-*g*-PEG surface binary pattern fabricated from PS 722. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank the Danish Agency for Science, Technology and Innovation (Innovation Consortium NANOBIONIC) for funding.

ABBREVIATIONS

BSA, bovine serum albumin; AFM, atomic force microscopy; FM, fluorescent microscopy; XPS, X-ray photoelectron spectroscopy; SPM, scanning probe microscopy; PVD, physical vapor deposition; PDA, polydopamine; PS, polystyrene; HCP, hexagonally close-packed; PLL-*g*-PEG, poly-*l*-lysine grafted poly(ethylene glycol)

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