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Protein immobilization and fluorescence quenching on polydopamine thin films

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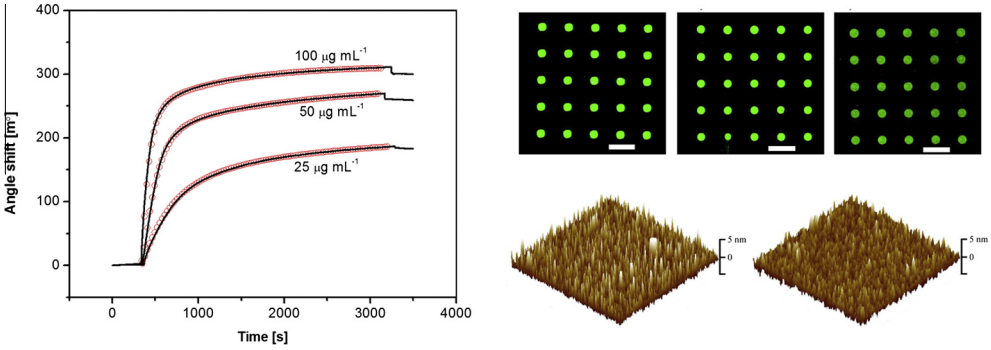
GRAPHICAL ABSTRACT



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ABSTRACT

Mussel inspired polydopamine (PDA) film has attracted great interest as a versatile functional coating for biomolecule immobilization in various bio-related devices. However, the details regarding the interaction between a protein and PDA film remain unclear. Particularly, there is very limited knowledge regarding the protein immobilization on PDA film, even though it is of essential importance in various fields. The situation is even more complicated if considering the fact that quite a number of approaches (e.g., different oxidizing reagent, buffer pH, grown time, grown media, etc.) have been developed to grow PDA films. In this work, protein attachment on PDA film was systematically investigated by using the real-time and label-free surface plasmon resonance (SPR) technique. The kinetics of protein-PDA interaction was explored and the influence of buffer pH and deposition media on the protein attachment was studied. Fluorescent protein microarray was further printed on PDA-coated glass slides for quantitative investigations and together with SPR data, the interesting fluorescence quenching phenomenon of PDA film was revealed. This work may deepen our understanding on the PDA-protein interaction and offer a valuable guide for efficient protein attachment on PDA film in various bio-related applications.

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1. Introduction

Interaction of proteins with a surface is implicated in various important biological activities. Understanding, and in turn controlling this interaction is essentially important for both fundamental research and practical applications [1]. Inspired by the adhesive protein of marine mussels for attachment to wet surfaces, polydopamine (PDA) film has been explored as a versatile surface coating to modify eventually all types of solid surfaces [2,3]. Due to the presence of functional groups such as indole, catechol, quinone and indolic/catecholic π-system, the PDA film possesses

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excellent adhesion stability to surface and also provides a versatile platform for further conjugation with other interesting species for surface functionalization and modification [2–8]. Particularly interesting is its ability to covalently bind with nucleophiles such as amines and sulfhydryls via Schiff's base formation or Michael addition, thus offering a universal strategy for protein attachment on solid surfaces [4,5,9].

Although PDA has been successfully used for protein attachment for years, the important details remain unclear regarding the interaction between protein and PDA film, for example, the conjugation density, interaction kinetics, immobilization stability, etc., which limits various practical applications. The situation is further complicated if considering the following three facts. (1) The oxidative polymerization mechanism of PDA is elusive at this time due to the complex redox processes involved and a series of intermediates generated during the reaction [10,11]. The exact chemical components of PDA are still under debate and therefore it is hard to figure out a clear picture about the protein-PDA interaction [12,13]; (2) A number of methods have been established to grow PDA films (different buffer, oxidant, polymerization duration, buffer pH, etc.); the film thickness, roughness, and chemical components may significantly vary with the synthetic conditions [3,14–17]; (3) the chemical components in the PDA film may also vary upon the environment change such as pH value due to the disturbed equilibriums of several reactive component pairs [11–13]. Therefore, it is particularly critical to investigate the protein-PDA interaction for fulfill the full potential of PDA film as a reactive while universal coating for protein attachment.

Considering the long-standing ambiguity on protein immobilization on PDA, in this work, we systematically studied the interaction of proteins with PDA film by using surface plasmon resonance (SPR) combined to fluorescent protein microarray. SPR is a powerful optical tool for study of protein-surface interactions with the inherent advantages of being label-free, real time, quantitative and sensitive [18–20]. On the other side, fluorescence is one of indispensable approaches in biological research, and particularly it is widely used for quantitative high-throughput detection, such as in fluorescent protein microarray [21,22]. In this work, we investigated the kinetics of protein-PDA interaction and studied the influence of deposition pH and deposition media of PDA film on the protein attachment. We also unveiled the compelling fluorescence quenching property of PDA film, which greatly depends on the film thickness. This work may help clarify the questions on protein-PDA interaction, and offer a useful guide for PDA's application involved biomolecules.

2. Experimental

2.1. Chemicals and materials

Dopamine hydrochloride, Tris(hydroxymethyl)aminomethane (Tris), and 0.01 M phosphate buffered saline (PBS, pH 7.4) were obtained from Sigma-Aldrich. Other chemicals and reagents were purchased from Aladdin (China). All chemicals were used as received without further purification. All solutions are prepared with deionized (DI) water from a Millipore water purification system with a resistivity of 18.2 MΩ cm. Tris buffer and phosphate buffer (PB) were used in these experiments and their pH value was adjusted if necessary.

2.2. Deposition of PDA films

PDA film was grown on SPR gold chip or standard glass slides by immersing the substrate into a freshly prepared PDA growing solution at ambient atmosphere for certain time. The PDA growing solution was prepared by dissolving 2.0 mg mL^{-1} dopamine hydrochloride in Tris buffer or PB buffer (pH 6.4, 7.4 and 8.5, adjusted with HCl or NaOH). It is worth noting that for PDA deposition on SPR chip, the growing duration is 20 min for pH 8.5 and 20 min × 3 (repeating three times) for pH 7.4 and 6.4. After the growth, the substrate was rinsed with DI water to remove the loosely absorbed PDA deposits and dried by gentle nitrogen flow for subsequent experiments.

2.3. SPR measurements

SPR measurements were conducted using a BI-4000 instrument (Biosensing Instruments, Tempe, AZ, USA), which is based on Kretschmann configuration and coupled with a monochromatic *p*-polarized laser (670 nm) as the light resource. The SPR chip was separated as two channels, namely, sensing channel and reference channel and the SPR signals from these two channels were simultaneously collected at a frequency of 10 Hz. All reported SPR data were obtained by subtracting the reference (background) signal from the sensing signal to eliminate the possible disturbance from environment. A flow injection system based on BI-DirectFlow™ Technology was used to deliver the sample solution onto the chip surface with near-zero dispersion. During the detection, 0.01 M PBS was first flowed through the sensing surface at a constant rate of 3.0 μL min^{-1} to obtain a stable baseline, followed by the injection of protein solution (in 0.01 M PBS) via a sample loop to the sensing surface at the same flowing rate, followed by rinsing with 0.01 M PBS buffer. The surface density of adsorbed proteins was calculated by the increase in the SPR response (micro-degree, m°) measured in PBS solution before and after protein solution injection.

2.4. Printing of protein microarray

Fluorescent protein microarrays were printed on PDA-coated glass slides by using Cy3-conjugated anti-Rabbit IgG was used as a fluorescent ink. The fluorescent protein was diluted with a printing buffer (0.01 M PBS with 10% glycerol and 0.005% Triton X-100) to a final concentration of 100 μg mL^{-1} and then printed with a Personal Arrayer™16 system (CapitalBio Corporation, Beijing, China) under contacting mode [21–23]. The surfactant Triton X-100 in the ink is able to suppress the coffee-ring effect for uniform protein attachment and its presence does not influence the stability of protein at the concentration used according to previous work [23–25].

After printing, the slides were kept in dark for overnight incubation and washed gently with TBST (0.05 M Tris, 0.138 M NaCl, 0.0027 M KCl and 0.05% Tween®20, pH 8.0) to remove unbound proteins. After drying, the slides were immediately imaged with LuxScan™ 10K-A Microarray Scanner (CapitalBio Corporation, Beijing, China) and the fluorescent images were obtained at 532 nm excitation. The obtained images were analysed to obtain the statistical fluorescent intensities.

2.5. Characterizations

X-ray photoelectron spectra (XPS) of PDA films deposited on silicon were collected by using an ESCALAB 250Xi system from Thermo Scientific. Atomic force microscopy (AFM) images were collected using a Nanoman AFM (Veeco metrology group, USA) in tapping mode.

3. Results and discussion

3.1. Thickness and roughness of PDA films

Various methods have been explored for growing PDA films on a wide variety of substrates and the film growth kinetics has also

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