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High-efficiency immunoassay platform with controllable surface roughness and oriented antibody immobilization

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Abstract: High-efficiency immunoassay platforms with controlled surface roughness (single- and dual-scale structured surface) were prepared by combining a facile layer-by-layer particle deposition approach with oriented immobilization of antibodies through boronic acid moieties. The as-prepared surfaces showed significantly enhanced antibody loading capacity and antigen recognition, as proven by the fluorescent images.

Fabricating high-efficiency immunoassay platforms has recently attracted much attention due to their vital roles in the early diagnosis for prevention and treatment of diseases.[1-4] Considerable efforts have been made to enhance detection sensitivity.[5-8] Antibodies are typically immobilized on solid surfaces to recognize the corresponding analytes by using specific recognition between antibody and its corresponding antigen. So, highly efficient surface immobilization with antibodies is essential for developing a high-sensitivity immunoassay. Generally, oriented immobilization of antibody has much higher antigen binding capability than those employing random covalent coupling or physical adsorption.[7] A primary reason is due to the oriented antibody immobilization makes antigen-binding domains of antibody more accessible to the related antigen, which greatly promotes the affinity of antibody-antigen binding. Boronic acid (BA) derivatives have received much attention as saccharide binders and cell sensors.[9-12] Recently, BA derivatives have shown potential in oriented immobilization of antibodies through the specific interaction between BA moieties and polysaccharides in the constant region (Fc) of antibody.[13-15] In our previous work,[14] we successfully prepared sulfobetaine-based polymer brush with BA moieties to obtain oriented immobilization of antibody and low bio-fouling surface. Compared with other site-specific antibody immobilizations, the oriented antibody immobilization based on BA derivatives demonstrated advantages such as uniform antibody orientation, high efficient antibody immobilization and mild reaction conditions.[16]

The antibody-loading amount on the surface is another key factor that can play an important role in the development of high-sensitivity immunoassay. Enlarging the surface area can increase the amount of immobilized antibody on a substrate, thus amplifying the detection signal of antibody-antigen recognition. As a common method, grafting polymeric brushes on substrates via surface-initiated polymerization has been widely employed to increase the surface anchor site for antibody immobilization.[18-21] Alternatively, various nanomaterials with large surface areas such as carbon nanotubes[22] and zinc oxide nanorods[23], have also been used to prepare nanostructured substrates for increasing antibody loading. However, a systematic investigation about the effect of the controllable roughness surface, in combination with oriented antibody immobilization, is still insufficient. Our current goals are focusing on the preparation of an immunoassay platform with both controllable surface roughness and oriented antibody immobilization on the surfaces. In particular, we will examine the impact of using single-scale and dual-scale structured surfaces on antibody immobilization and subsequent antigen recognition. Compared with a flat surface, a single-scale structured surface would produce much greater surface roughness, and the surface roughness on a dual-scale structured surface would be even larger (details in ESI, Fig. S1/S2), potentially allowing immobilization of much larger amounts of antibody than a flat surface.

We first prepared single- and dual-scale structured surfaces with different surface roughness via layer-by-layer (LbL) particle deposition (Fig.1). An epoxy coating of about 50% conversion on a glass slide was prepared, followed by deposition of 1000 nm particles via spin-coating.[24,25] After the epoxy coating was fully cured, the robust single-scale structured coating was obtained. The single-scale sample was subsequently treated with 3-aminopropyl triethoxysilane (APS) and hydrochloric acid (HCl) to render the surface positively charged, followed by electrostatic deposition of 100 nm particles to obtain the dual-scale structured coating. To enhance its mechanical property, the silica particles on the structured surfaces were further consolidated by reacting with $SiCl_4$. As shown in the scanning electron microscope (SEM) image (Fig. 2), the large particles (~1000 nm) were randomly distributed in a non-close packing pattern to form the single-scale structured surface. The dual-scale structured surfaces consisted of closely packed small particles (~100 nm) on the large particles.

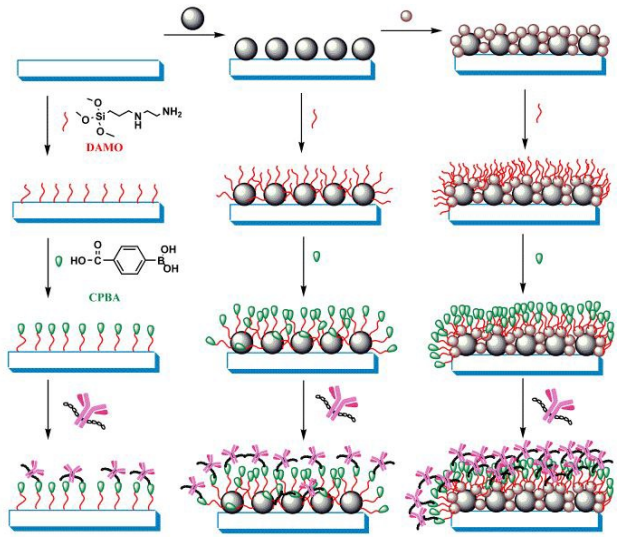

Fig. 1 Schematic of the flat (left), single-scale (middle) and dual-scale (right) structured surfaces for oriented immobilization of antibodies.

The surface roughness of the samples was examined by atom force microscopy (AFM) (Fig. S3). The root mean square (RMS) roughness of the single-scale and dual-scale structured surfaces was respectively increased to 140 ± 40 nm and 245 ± 30 nm, significantly greater than the flat reference (3 ± 1 nm). It should be pointed out that the roughness for the structured coatings obtained from AFM was likely underestimated due to the dimension of the AFM tip.[26]

Hereafter, the samples were first modified with [3-(2-aminoethyl) aminopropyl] trimethoxysilane (AAPTS), followed by the introduction of 4-carboxyphenylboronic acid (CPBA) via the activation of carboxylic groups with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). The CPBA-modified flat, single and dual-scale structured samples were respectively denoted as V-CPBA, S-CPBA and D-CPBA. In ATR-FTIR spectra, a new amide structure peak (at 1565 cm^{-1}) was observed on the modified samples, confirming the successful condensation reaction between AAPTS and CPBA on the surfaces (Fig. S4). The surface grafting of BA moiety was also confirmed by X-ray photoelectron spectroscopy (XPS) (Fig. S5).

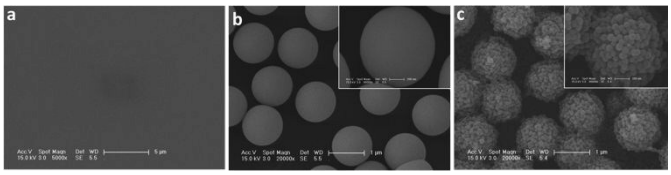

Fig. 2 SEM images of (a) flat reference, (b) single-scale structured surface and (c) dual-scale structured surface.

To investigate the effects of surface roughness on the solid phase immunoassay performance, FITC-labelled human serum albumin antibody (FITC-HSA-Ab) was used as a typical antibody to evaluate the amount of antibodies immobilized on the different samples. The related fluorescent images were collected by confocal microscopy, and the fluorescence intensities were quantitatively analysed by using Image Pro software. To prevent nonspecific adsorption of antibody on surfaces, all samples were incubated in 0.5% polyoxyethylene sorbitan monolaurate (Tween-20) solution at 37 °C for 1 h before FITC-HSA-Ab immobilization.

As shown in Fig. 3, significantly different fluorescence intensities with different concentrations of FITC-HSA-Ab were observed on these samples, which directly reflected the amount of immobilized antibody on the surfaces. The representative fluorescence images of the antibody-immobilized samples were also provided in Fig. S6. Low fluorescence intensities were detected on the flat V-CPBA reference regardless of antibody concentration, which is mainly attributed to the low CPBA density on the surface. This is likely the consequence of the low surface area as well as the two-dimensional antibody immobilization on a flat surface. In contrast, the fluorescence intensities of the structured (single- and dual-scale) surfaces were much stronger, even at a low FITC-HSA-Ab concentration (10 ng/mL), and those intensities increased markedly with increasing the antibody concentration. In addition, the amount of antibody immobilized on the surfaces can be well adjusted by changing antibody concentration in the solution. The dual-scale structured surfaces exhibited the highest fluorescence intensity, and the difference of fluorescence intensity between the single and dual-scale structured samples became even larger with increased antibody concentrations, which can be attributed to the even larger surface roughness of the dual-scale structured surface. Generally, the amount of immobilized antibody on the hierarchically structured samples was about 3 orders of magnitude larger than on the V-CPBA reference, which can be mainly attributed to the increased amount of CPBA immobilized on the surface with high roughness, leading to the increased amounts of the chemically immobilized antibody on the surfaces.

As shown in our previous study,[24] structured surfaces might often show higher non-specific protein adsorption (1.5 to 2.0-fold greater than a flat surface), which could reduce the signal-to-noise (S/N) ratio in immunoassay detection. To eliminate the influence of background signals, all samples were blocked with 5% skim milk powder in PBS for 1 h at 37 °C before antigen detection.[27]

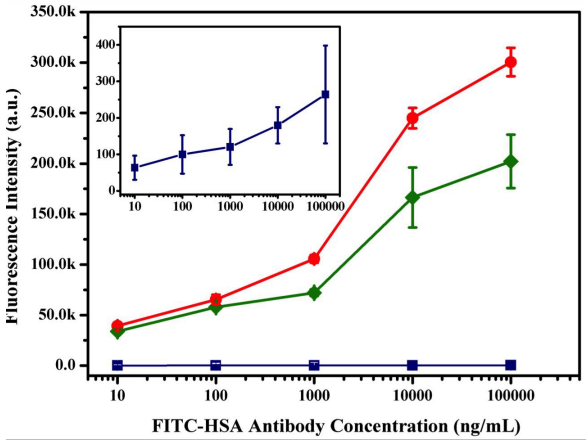

Fig. 3 The amount of fixed FITC labelled antibody on the CPBA-modified samples of (■) flat reference, (●) single-scale structured surface, (▲) dual-scale structured surface. Inset: magnification fluorescence intensity of immobilized antibody on flat reference.

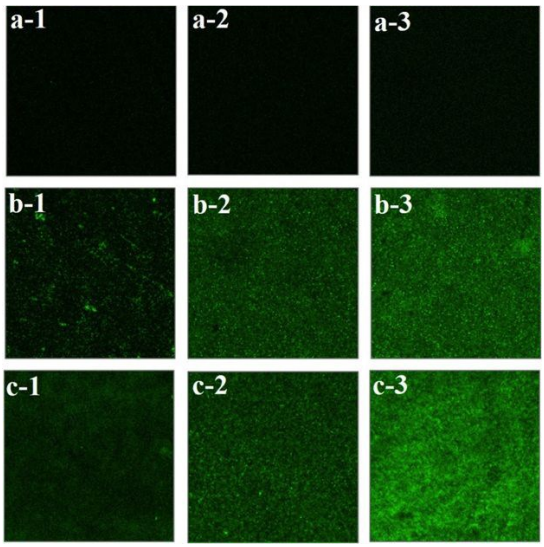

Fig. 4 Representative fluorescence images of the samples after recognizing antigen at different antigen concentrations (0.01, 10 and 10000 pg/mL for 1, 2 and 3, respectively). (a) V-CPBA, (b) S-CPBA surface, (c) D-CPBA surface.

Once blocked by the skim milk solution, the antibody-immobilized samples (incubated in 10^5 ng/mL antibody solution) were used for recognition of FITC-labelled antigens. The representative fluorescence images of the samples were immediately recorded after 2 h incubation in the antigen solutions (Fig. 4). Qualitatively, the D-CPBA surfaces showed remarkable sensitivity of antigen detection, even with a relatively low detection limit of 0.01 pg/mL (Fig. 5), which is much lower than

the previously reported results.[28,29] The sample S-CPBA also showed major improvement in antigen detection with respect to the flat control, but the fluorescent intensity was not as high as on the D-CPBA sample, especially at high antigen concentrations. The results clearly indicated that the increased surface roughness led to the remarkable increase of antibody loading, which played a crucial role in the preparation of an efficient antigen-recognition surface. The high sensitivity of our immunoassay platform was attributed to the following two aspects: first, the total amount of immobilized antibodies was dramatically increased through increasing the surface roughness; second, the accessibility of antigens to antibodies was highly enhanced due to oriented antibody immobilization.

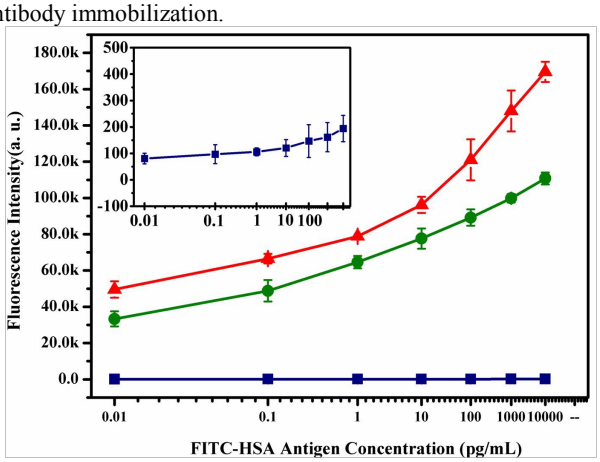

Fig. 5 FITC-HAS antigen recognition for the CPBA-modified samples of (■) flat reference, (●) single-scale structured surface, and (▲) dual-scale structured surface. Inset: magnification of fluorescence intensity on flat reference.

In conclusion, we prepared high-efficiency immunoassay platforms by combining high-roughness surface with oriented antibody immobilization through boronic acid moieties. The amount of immobilized antibodies was greatly improved with the increased surface roughness, about 3-4 orders of magnitude larger than on a flat reference. The structured samples, especially the dual-scale structured surface, showed extremely high detection sensitivity for the FITC-labelled antigens. Due to its efficiency, this type of immunoassay platform may become an attractive strategy for diverse bioassays.

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† Electronic Supplementary Information (ESI) available: Details of synthesis of silica particles, fabrication of the single-scale and dual-scale structured surfaces, preparation of the AAPTS- and CPBA-functionalized surfaces, the oriented immobilization of HSA-Ab, the recognition of HSA-Ag, water contact angle data, AFM images, FTIR-ATR spectra, high-resolution B_{1S} spectra, representative fluorescence images of the FITC-labeled antibody-immobilized samples. See DOI: 10.1039/b000000x/

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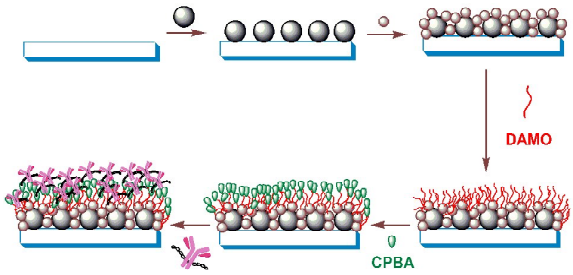

High-efficiency immunoassay platforms were facilely prepared by combining a layer-by-layer particle deposition with site-specific antibody immobilization through boronic acid moieties.