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Phenylboronic acid polymer brush-enabled oriented and high density antibody immobilization for sensitive microarray immunoassay

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ABSTRACT

There still has a big challenge for low-abundance protein detection with antibody microarrays. In this work, phenylboronic acid (PBA) polymer brush-enabled oriented, high density, and covalent antibody immobilization was realized for sensitive antibody microarrays. PBA-enabled oriented antibody attachment via carbohydrate at Fc portion keeps antigen-binding sites fully expose to their corresponding antigens, resulting in higher antibody–antigen (Ab–Ag) binding efficiency. Sandwich immunoassay with rabbit IgG as model analyte was performed on poly(glycidyl methacrylate)-amino-phenylboronic acid-coated glass slide (PGMA-APBA-slide). One order improvement of LOD was achieved as compared with that on poly(glycidyl methacrylate) glass slide (PGMA-slide). The improvement is mainly attributed to PBA-assisted high density and oriented antibody immobilization. This work provides a versatile and effective strategy to develop high sensitive antibody microarrays for low-abundance protein analysis in various proteomic applications.

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1. Introduction

Antibody microarray has emerged as a promising tool for rapid, high-throughput, and miniaturized protein analysis [1,2]. It has been widely used in various fields including fundamental life science, biomedical diagnosis, drug discovery and food safety detection etc. [3–6]. Despite intense efforts that have been dedicated to antibody microarray, there still have many challenges to detect low-abundance protein due to the lack of “PCR-like” technique for protein duplication, severely limiting its further applications, especially in early diagnosis of fatal diseases (i.e. cancers) [7–9].

Antibody molecules have a roughly Y-shaped structure. The antigen-binding domains are located at the top of each of the two outstretched arms. Oriented immobilization of capture (primary) antibody is of very critical in heterogeneous immunoassays [10]. Oriented immobilization can fully keep its antigen-binding sites expose to paired antigen, thus significantly improving antibody–antigen binding efficiency and detection sensitivity [11–13]. It is one of the most critical issues to efficiently immobilize capture (primary) antibody for development of highly sensitive antibody microarray [14,15]. In addition, since the diverse physical and chemical properties of proteins (antibody and analyte), severe nonspecific adsorption usually occurs in microarray immunoassays, resulting in low signal-to-noise ratio and poor sensitivity [16,17]. Ideally, antibody should be homogeneously immobilized on a substrate with uniform orientation, high density and stability, while nonspecific adsorption should be minimized during the whole assay process.

Various strategies have been developed for antibody immobilization in the past decade [18–20]. Generally, these methods can be divided into two main categories: non-oriented immobilization through direct physical adsorption and covalent coupling through amine group on antibody [21]; oriented anchoring via protein A/G–Fc portion interaction, avidin/streptavidin–biotin, oxidation of carbohydrate located at Fc portion, and production of mercapto group at the end of Fab. However, specially chemical or biological modifications of both antibody probe and chip substrate are required by all these strategies for oriented antibody attachment. These modifications are not only time consuming and costly, but decrease the repeatability and reliability of antibody array chips. Therefore, a versatile and effective approach for oriented and high density antibody immobilization is urgently needed to develop high performance antibody microarray. It has been reported that boronic acid can react with the 1,2- or 1,3-diol of saccharide to form a stable cyclic boronate ester at room temperature [22]. Thus, the

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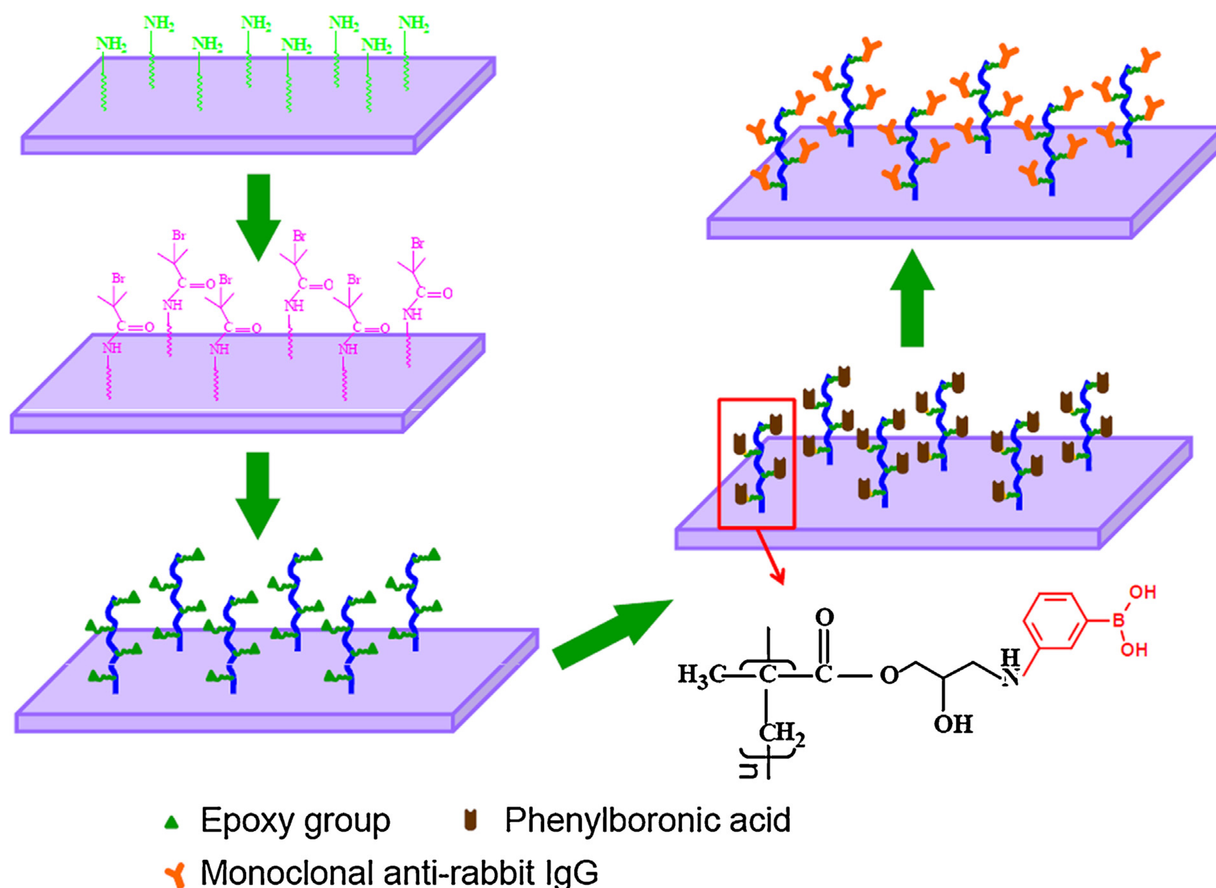

Scheme 1. Schematic illustration of PGMA-APBA-slide preparation and oriented antibody immobilization.

formation of boronate ester between boronic acid and carbohydrate located at Fc portion could be an alternative approach for oriented antibody immobilization. In this work, a 3-D phenylboronic acid polymer brush-functionalized glass substrate was developed to realize oriented, covalent, and high density antibody anchoring [23–25]. This method takes the advantages of copolymer brush (i.e. 3-D and flexibility) and the boronic acid. In addition, no chemical or biological modifications are applied to antibody probe in this strategy. Improved performance of antibody microarray was achieved on PGMA-APBA-slide, demonstrating its great potential for development of high sensitive antibody microarray in practical applications.

2. Experimental

2.1. Materials and chemicals

Monoclonal anti-rabbit IgG, rabbit IgG, Cy3-labeled goat anti-rabbit IgG, Cy3-labeled rabbit anti-goat IgG, tris buffered saline (TBS, pH 8.0) with 0.05% Tween20, carbonate buffered saline (CBS, pH 9.6), phosphate buffered saline (PBS, pH 7.4), (3-aminopropyl) triethoxysilane (APTES, 99%), glycidyl methacrylate (GMA, 97%), 2-bromoisobutyryl bromide (BIB, 98%), triethyl-amine (TEA, 99.5%), copper(II) bromide ($CuBr_2$, 99.999%), 2,2′-bipyridyl (Bipy, ≥99%), L-ascorbic acid, 3-aminophenylboronic acid (APBA), and dextran (M_W ~ 6000) were ordered from Sigma-Aldrich (Shanghai, China). Albumin from bovine serum (BSA), β-cyclodextrin (98%), and carboxylated chitosan (M_W ~ 15,000, carboxylation degree ≥ 60%) were bought from Aladdin (Shanghai, China). D-fructose and D-maltose were purchased from Chengdu Kelong chemical reagent company. D-Glucose was received from Hengxing Chemical (Tianjin).

2.2. Preparation of initiator-activated glass substrate

Microscope glass slides with dimensions of 75 mm × 25 mm × 1 mm were cleaned with alcohol for 10 min under sonication and rinsed with deionized (DI) water. The pre-cleaned slides were treated overnight by 1 M potassium hydroxide (KOH) solution at room temperature to remove organic residues and to promote hydroxylation. These slides were rinsed carefully for 3 times with DI water, followed by drying with N_2 flow. Subsequently, hydroxylized slides were silanized by treatment with 3% (V/V) APTES alcohol solution for 2 h at room temperature. The silanized slides were intensively rinsed with alcohol and DI water, followed by drying with N_2 stream and baking at 110 °C for 150 min.

Initiator was covalently conjugated on APTES-modified slide (Scheme 1a). Specifically, the APTES-modified slides were immersed into an ice-cold tetrahydrofuran (THF, 20 mL)/triethylamine (TEA, 154 μL) mixture, to which 128 μL of initiator 2-bromoisobutyryl bromide (BIB) was then added dropwise. The reaction was kept at 0 °C for 15 min to minimize the evaporation of BIB and then at room temperature for 2 h. The slides were thoroughly washed with alcohol and DI water, followed by flow drying with N_2.

2.3. Preparation of PGMA-slide and PGMA-APBA-slide

The GMA polymer brush on slide was synthesized following the procedure reported elsewhere [26,27]. In detail, initiator-activated slides were put in 20 mL of methanol/water (1:1, V/V) containing 2,2′-bipyridyl (Bipy, 4.6 mg mL^{-1}), $CuBr_2$ (3.35 mg mL^{-1}) and GMA (2%, V/V). Before polymerization, given amount of ascorbic acid was rapidly added into the as-prepared polymerization solution. Polymerization was performed in a closed reaction vessels

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