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Paper based colorimetric biosensing platform utilizing cross-linked siloxane as probe

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ABSTRACT

Paper based colorimetric biosensing platform utilizing cross-linked siloxane 3-aminopropyltriethoxysilane (APTMS) as probe was developed for the detection of a broad range of targets including H_2O_2, glucose and protein biomarker. APTMS was extensively used for the modification of filter papers to develop paper based analytical devices. We discovered when APTMS was cross-linked with glutaraldehyde (GA), the resulting complex (APTMS–GA) displays brick-red color, and a visual color change was observed when the complex reacted with H_2O_2. By integrating the APTMS–GA complex with filter paper, the modified paper enables quantitative detection of H_2O_2 through the monitoring of the color intensity change of the paper via software Image J. Then, with the immobilization of glucose oxidase (GOx) onto the modified paper, glucose can be detected through the detection of enzymatically generated H_2O_2. For protein biomarker prostate specific antigen (PSA) assay, we immobilized capture, not captured anti-PSA antibody (Ab_1) onto the paper surface and using GOx modified gold nanorod (GNR) as detection anti-PSA antibody (Ab_2) label. The detection of PSA was also achieved via the liberated H_2O_2 when the GOx label reacted with glucose. The results demonstrated the possibility of this paper based sensor for the detection of different analytes with wide linear range. The low cost and simplicity of this paper based sensor could be developed for "point-of-care" analysis and find wide application in different areas.

1. Introduction

Recently, paper based analytical devices have gained great interest due to their attractive advantages, such as low cost and simplicity (Noiphung et al., 2013; Noor and Krull, 2013; Pozuelo et al., 2013). It is believed to be one of the least expensive platforms available for developing assays (Martinez et al., 2007). The paper substrates have porous structure, which could facilitate the immobilization of sensing materials and the diffusion of analytes. Lateral flow systems based on papers have been widely used for qualitative and semiquantitative analyses (Lopez-Marzo et al., 2013; Mei et al., 2013; Zhu et al., 2013). Many new applications on this area are still being developed, for example, Whitesides et al. reported 3D paper based microfluidic devices, which combine the capabilities of conventional microfluidic devices with the simplicity of paper based tests and require only small volumes of fluid (Martinez et al., 2010; Vella et al., 2012).

Compared to the conventional analytical methods that require sophisticated instruments, these paper based analytical devices are usually integrated with simple colorimetric detection systems (Hossain and Brennan, 2011; Jokerst et al., 2012; Xu et al., 2011). The level of target analyte is quantified through the color change. For example, some of the sensors can achieve the detection of analytes through naked eyes, while others with the aid of a scanner or even camera-equipped cell phone (Alkasir et al., 2012; Martinez et al., 2008; Yildiz et al., 2013). The picture taken by the scanner or cell phone can then be interpreted into quantitative analtye concentrations by software. Such sensors are disposable, easy to operate and can be used for on-site analysis, which have potential to be developed into 'point-of-care' analytical devices and have wide applications in undeveloped countries (Wang et al., 2012, 2013; Yan et al., 2012).

Various paper based colorimetric sensors have been developed for the testing of different target analytes, such as glucose, protein biomarkers and DNA sequences (Cheng et al., 2010; Yetisen et al., 2013; Yuan et al., 2012). Different dyes and nanomaterials have been selected as colorimetric indicator and adapted onto papers to develop biosensing platform. The detections are based on target analyte induced color change of the dyes or nanomaterials on the paper surface.

In this work, when working on the paper based biosensors, we first treated the filter paper with siloxane 3-aminopropyltriethoxysilane (APTMS) to facilitate the following modification of the filter paper.

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We found out when APTMS was cross-linked with glutaraldehyde, it was turned into brick-red color. The resulting complex (APTMS–GA) can then be oxidized by H_2O_2, leading to an obvious color change. Based on this phenomenon, we applied the complex onto filter paper and developed a new paper based colorimetric biosensing platform for the detection of three different analytes, H_2O_2, glucose and protein biomarker prostate specific antigen (PSA). Initial studies were conducted to determine the feasibility of the assay on paper for H_2O_2 detection. Different concentrations of H_2O_2 resulted in the variation of color intensity of the paper, with can be quantified with the software of Image J. Next, after the immobilization of glucose oxidase (GOx) onto paper, we applied the assay for glucose detection. Finally, we investigated the possibility of the assay for PSA detection with the immobilization of capture-anti PSA antibody (Ab_1) onto paper and using GOx as detection antibody (Ab_2) label.

2. Experimental methods

2.1. Apparatus and reagents

Prostate specific antigen (PSA) and anti-PSA antibody were obtained from Dingguo Biotechnology Co., Ltd. (Beijing, China). Glucose oxidase (GOx, from *Aspergillus niger*, EC 1.1.3.4.150000 units g^{-1}), 3-aminopropyltriethoxysilane (APTMS) and chitosan (MW = 140,000 –220,000) were purchased from Sigma-Aldrich. All other reagents were of analytical grade and deionized water (MillQ, 18.2 MΩ) was used throughout the study. Scanning electron microscope (SEM) images were obtained from Nova NanoSEM230 (FEI, USA).

2.2. Preparation of APTMS modified filter paper

Disk-shaped filter paper was cut into small disks with a hole puncher, each with a diameter of 6 mm. Then the small disks were soaked for 5 min in 5% of APTMS in ethanol. After drying, 7.5 μL of 0.25% glutaraldehyde (GA) was dropped onto the papers to form the APTMS–GA matrix. Then the prepared papers were ready for H_2O_2 detection. For H_2O_2 detection, APTMS–GA modified papers were immersed into solutions of varying concentrations of H_2O_2 for 5 min. After drying, the color intensity of the papers was measured after taking photos and then interpreted by software Image J. The obtained color intensity was used to construct the calibration curve.

2.3. Preparation of glucose biosensor based on the filter paper

For glucose detection, 10 μL of 0.5% chitosan solution was first added onto the APTMS–GA modified filter paper surface. After drying, 0.25% GA solution was dropped onto the paper for 30 min. Then 10 μL of GOx solution (15 mg/mL) was added onto the APTMS–GA modified filter paper surface. After drying and washing, the filter papers were then immersed into solutions of varying concentrations of glucose for 10 min and the color intensity of the papers was measured.

2.4. Preparation of immunosensor for the detection of PSA based on the filter paper

The immunosensor based on the filter paper for the detection of PSA was constructed utilizing the traditional sandwich structure. GOx modified gold nanorod (GNR) was prepared as detection anti-PSA antibody (Ab_2) label. First, GNR was synthesized according to the reference report with minor revision (Nikoobakht and El-Sayed, 2003). Gold seed solution was prepared by reduction of $HAuCl_4$ solution with $NaBH_4$. Then, to prepare GNR, into 50 mL of 0.1 M CTAB solution, 1.1 mL of $HAuCl_4$ (1% w/w), 0.4 mL of $AgNO_3$ (10 mM), and 0.29 mL of ascorbic acid (0.1 M) were added sequentially. After stirring, the solution turned colorless. Finally, 0.06 mL of the above synthesized gold seed solution was added and the color of the solution changed gradually after 10 min. The solution was kept for 24 h and then centrifuged to obtain GNR.

To conjugate GOx and Ab_2 onto GNR, GNR was first dispersed into 0.02 M cysteine solution for 2 h to form the self-assembled monolayer onto GNR. Then, the modified GNR was added into 0.25% of glutaraldehyde for 1 h. After centrifugation, the GNR was dispersed into solution containing 2 mg/mL of GOx and 0.2 mg/mL of Ab_2 for 1 h. GNR-GOx-Ab_2 was then obtained after another round of centrifuge.

Gold nanoparticles (AuNPs) of 13 nm diameter were synthesized and used for the adsorption of primary anti-PSA antibody (Ab_1). Typically, a 250-mL aqueous solution containing 100 mL of 0.01% (w/w) $HAuCl_4$ was brought to a vigorous boil, then 2 mL of trisodium citrate (2%) was added rapidly to the solution. The solution was boiled for another 15 min, and the color changed from pale yellow to deep red. The solution was cooled to room temperature with continuous stirring.

To construct the immunosensor, 10 μL of 0.5% chitosan solution was dropped onto APTMS–GA modified filter paper surface. After drying, AuNPs were adsorbed onto the paper surface for the following adsorption of Ab_1. Then, the filter paper was blocked with 1% BSA solution and incubated with different concentrations of PSA for 1 h (37 °C). After washing extensively, the prepared GNR-GOx-Ab_2 was added onto filter paper surface and incubated for another 1 h (37 °C). Finally, after the construction of the paper based immunosensor, 10 μL of glucose solution (10 mM) was applied onto the paper surface. After 10 min, the color intensity of the papers was measured.

2.5. Color intensity measurement

After taking photos, the color intensity of the filter paper in the photo was quantified and analyzed using Image J software. To measure the signal from each paper, a circle was used as a uniform region of interest, which covers roughly 90% of the surface of paper. The signal for the individual paper was calculated as average of the intensity values of the respective papers.

3. Results and discussion

3.1. Fabrication of the APTMS modified paper for H_2O_2 detection

Initially, we studied the reaction of APTMS with GA. Fig. 1A shows the schematic representation of the structure of APTMS and its reaction with GA. Fig. 1B displays the solution of APTMS (5% v/v in ethanol, 500 μL), which is a colorless solution. After the addition of 500 μL of GA (0.25%) into APTMS, the color of the solution turned into brick-red instantly. When APTMS was mixed with GA, the amino groups on APTMS reacted with GA to form Schiff bases and led to the formation of APTMS dimer, which displays brick-red color (Migneault et al., 2004). Then, the reaction of the APTMS–GA complex with H_2O_2 was investigated. With the addition of 200 μL of 1 M of H_2O_2 into APTMS–GA, it can be seen that the solution turned into colorless again, demonstrating that the APTMS–GA complex was oxidized by H_2O_2. The oxidation reaction may destroy the Schiff bases formed between APTMS and GA, breaking the dimer to APTMS monomer again, and then the color was faded. No color change was observed in the absence of APTMS or GA for the concentration of H_2O_2 tested.

After proving the reaction of H_2O_2 with APTMS–GA could induce the color change of the APTMS–GA in solution, we then tested the assay on filter paper. APTMS was extensively utilized to