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Sensitive "capillary ELISA" via vapor-phase surface modification

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ABSTRACT

Sensitive ELISA for C-reactive protein (CRP) was performed in a capillary tube based on simple vapor-phase surface chemistry. Amine surfaces were produced in capillary tubes in two different methods using 3-aminopropyltriethoxysilane (APTES) in liquid and vapor phases. Those amine surfaces were tethered with gold nanoparticles (AuNPs) to be characterized by field emission scanning electron microscopy (FE-SEM) resulting in more dense and dispersed AuNP populations from the vapor-phase surface. Feasibility of a miniaturized home-made optical detector for "capillary ELISA" was performed by infusing into capillary tubes the colored solutions that had just gone through ELISA on a 96-well plate. The compatibility of the two results obtained from ELISAs on a well-plate and in capillary tubes, confirmed dependability of the miniaturized detector. Finally "capillary ELISA" was performed on the home-made detector using anti-CRP immobilized capillary tubes that had been prepared via vapor-phase surface treatment, resulting in detectability of 1.0 ng/mL of CRP, which turned out to be around 10 times higher in sensitivity than that of the well-plate ELISA.

1. Introduction

Recently need for a simple and accurate diagnostic method has been causing ever increasing number of researches in the fields of point-of-care (POC) diagnostics [1–5]. Mostly the working principle of those POC researches is based on the traditional ELISA method with the aim of simplifying the complex processes with low cost. Until now lateral-flow assay (LFA) system based mainly on paper material is understood to be the most successful system due to low cost and installibility of all the functional features required in ELISA in a simple controlled-flow system [4,5]. However limitation in detection capability is reported to be the major barrier to the widespread use of the LFA for POC applications.

Capillary tube has been widely used as an indispensable component in many analytical systems including gas chromatography (GC) [6], liquid chromatography (LC) [7,8] and gel permeation chromatography (GPC) [9]. Also in the POC applications, various versions of capillary tube-based immunoassay systems were developed taking advantage of its simple structural feature [10–25]. Rectangular-shaped capillary tubes were used to perform enzyme immunoassay for various types of biomarkers [18,22–24]. When the well-known surface chemistry based on 3-aminoprolytriethoxysilane (APTES) and glutaraldehyde (GA) were used to install antibody in the capillary tube, the immunoassay results produced 10 ng/mL level detection capability [24]. When the inner surface of the rectangular glass was equipped with multi-layered functional coatings, high degree of detectability was able to be produced with limit of detection (LOD) of 0.1 ng/mL [18]. However this method required complex steps to prepare multi-layered functional coatings.

In this report we present a highly sensitive immunoassay in a capillary tube [13,15,26] based on the well-known simple surface-chemistry that uses APTES and GA for immobilization of antibody. In particular treatment of APTES was processed in vapor phase. C-reactive protein (CRP) was chosen as a target analyte, which has been reported to be a biomarker for coronary artery disease [27] and inflammation [28]. With inner diameter of 0.4 mm and length of 32 mm, the total inner volume (V_T) of 5 μL is an advantage requiring minimal amount of expensive biological molecules. Detection of the "capillary ELISA" was processed by a miniaturized home-made optical system based on colorimetric analysis using 3,3′,5,5′-tetramethylbenzidine (TMB) as a substrate. Simple procedure for surface modification and very sensitive detection of the "capillary ELISA" may be extended to POC applications.

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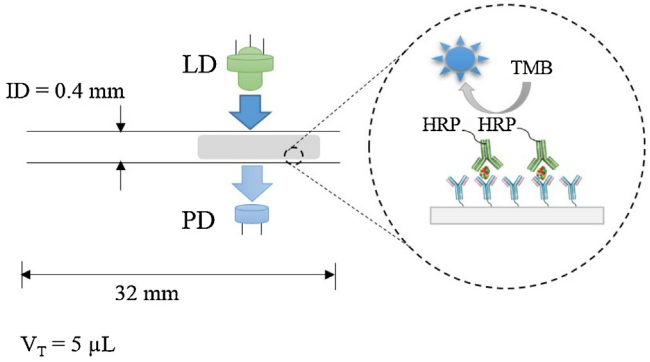

Fig. 1. Figurative description of the general working concept of the "capillary ELISA". (For interpretation of the references to color in the text, the reader is referred to the web version of this article.)

2. Experimental

2.1. Materials, chemical reagents, and equipment

The 10 nm gold nanoparticles (NPs) were purchased from BBI International (Ted Pella, Redding, CA; number of particles 5.7E12, optical density 0.75@520 nm, and 9.46 nM). Capture anti-C-reactive protein (anti-CRP) (4C28-C5), detection anti-CRP (4C28-CRP135), and CRP (8C72) were purchased from HyTest Ltd. Glutaraldehyde solution (GA, 25% in H_2O, G5882), 3-aminopropyltriethoxysilane (APTES, 440140), 3,3′,5,5′-tetramethylbenzidine (TMB, T0440-100ML) and bovine berum albumin (BSA, A7030-50G) were purchased from Sigma-Aldrich. Horse radish peroxidase (HRP) conjugation kit (ab102890) was purchased from Abcam. Blocker™ Casein (37528) and 20xPBS Tween-20 (28352) were purchased from Thermo Fisher Scientific (Nunc). Capillary tubes (cat. no. 1-000-0050, capacity 5 μL, length 32 mm, OD 0.95, and ID 0.4 mm) were purchased from Drummond Scientific Co. Well plates (96-well, polystyrene) were purchased from Thermo Fisher Scientific (Nunc). While the immunoassays performed in ELISA well-plated were measured by TECAN Group Ltd. (Infinite 200 PRO), the immunoassays carried out in capillary tubes were measured by a miniaturized home-made optical detection system. Deionized (DI) water was purified using a Milli-Q water purification system. Images of the field emission scanning electron microscopy (FE-SEM) were collected on a FEI sirion-400 FE-SEM.

2.2. Preparation of HRP-conjugated CRP antibody (anti-CRP•HRP)

Conjugation of HRP to anti-CRP was processed by a HRP conjugation kit (ab102890) by following the protocol provided in the kit. As described in the protocol, 10 μL of CRP antibody (1 mg/mL) (4C28-CRP135) was added to 10 μL of the "modifier" (biotin-NHS) to be incubated for 10 min. "HRP mix" (HRP-avidin) was mixed with 100 μL of PBS (1×) to be added to the mixture solution of the modifier and anti-CRP. After incubating the mixture solution for 3 h at room temperature, 1 μL of the "quencher reagent" was added and left for 30 min.

2.3. ELISA on a 96-well plate

Monoclonal anti-CRP was mixed with coating buffer (0.1 M $NaHCO_3$) to be put into each well of a 96-well plate by 50 μL with concentration of 10 μL/mL. After incubation at 4 °C overnight, the well plate was washed with 0.01% Tween–20 (1xPBS, pH 7.4) five times. Each well was added with 200 μL of Blocker™ Casein, incubated for 30 min and washed with 0.01% Tween–20 (1xPBS, pH 7.4) five times. Each well was added with varying amount of CRP (0, 0.1, 0.5, 1, 5, 10, 100 and 1000 ng/mL), incubated for 30 min and washed with 0.01% Tween–20 (1xPBS, pH 7.4) five times. Into each well 50 μL of anti-CRP•HRP (5 μg/mL), which had been prepared as described in Section 2.2, was added to be incubated for 30 min followed by washing with 0.01% Tween–20 (1xPBS, pH 7.4) five times. TMB solution (50 μL) was added into each well. After incubating 20 min the color development was analyzed by the TECAN reader.

2.4. Preparation of gold nanoparticles (AuNPs) conjugated with anti-CRP (anti-CRP •AuNPs)

AuNPs conjugated with anti-CRP (4C28-CRP135) were prepared by following the procedure described previously [29,30]. A 9.46 nM solution of AuNPs (10 nm diameter, 1 mL) was mixed with 20 μL of 40 mM potassium carbonate (K_2CO_3) by keeping pH of the AuNPs solution at around 9. The reaction mixture was shaken for 1 min. After adding 100 μL of CRP antibody, the solution was again shaken for 5 min, followed by adding 100 μL of 10% BSA in DI water. The mixture solution was incubated for 1 h. In order the remove unbound antibodies and BSA, the solution was centrifuged at 12,000 rpm for 20 min. The precipitate was collected to be dissolved in 1 mL of 50 mM PBS (pH 8.0, 0.1% NaN_3).

2.5. Immobilization of capture anti-CRPs in a capillary tube

In order to install capture anti-CRPs inside a capillary tube, the glass tube was treated with oxygen plasma for 300 s (30 Pa, 100 mL/min of oxygen and 100 W). The immobilization of the anti-CRP was processed in gas phase using only half part of the capillary tube. The instrumental set-up for the vapor-phase reaction is described in the Supporting information [S1]. In brief, 2 mL of APTES in a round bottom flask was carried over to micro-capillary tubes via aplastic tubes by heating the flask at 120 °C for 20 min. The capillary tubes were then imbued with mixture solution of glutaraldehyde (aqueous 25 wt.%) and sodium cyanoborohydride ($NaBH_3CN$) (1.0 wt.% to the total reaction mixture). The micro-capillary tubes filled with the reaction mixture were incubated for 4 h at room temperature to be washed with DI water. Now the aldehyde-functionalized surface was reacted with capture anti-CRP (2.5 μL, 10 μg/mL) to be incubated overnight at 4 °C. Now the capillary tubes immobilized with capture anti-CRP were washed with 0.01% Tween–20 (1xPBS, pH 7.4). The capillary tubes were imbued with 5 μL of blocker casein to be incubated for 30 min, followed by washing with 0.01% Tween–20 (1xPBS, pH 7.4).

2.6. Use of AuNPs to examine the amine functionalized surface and the immunoassay

In order to compare the efficiency of two different amination processes in capillary tubes, AuNPs were tethered on the two amine surfaces that had been prepared by in liquid-phase and vapor-phase. The vapor-phase amination was processed as described above. Liquid phase amination was processed by treating capillary tubes with oxygen plasma followed by immersing the tube in ethanol containing 0.1% of APTES for 30 min. After washing the capillary tubes with ethanol a few times the capillary tubes were baked for 10 min at 120 °C. In order to functionalize AuNPs on those amine surfaces, 5 μL of AuNPs (9 nM, 10 nm) was infiltrated into the amine functionalized capillary tubes to be incubated for an hour followed by washing with DI water. The incubated capillary tubes were broken into small pieces to be characterized by FE-SEM. Also, in an effort to confirm the viability of the vapor-phase amination in a capillary tube, sandwich-type immunoassays were carried on the vapor-phase amine surfaces using anti-CRP•AuNP so that the sandwich-type product may be characterized by SEM