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Paper-based electrochemiluminescence origami device for protein detection using assembled cascade DNA–carbon dots nanotags based on rolling circle amplification

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ABSTRACT

In this work, we developed a cascade signal amplification strategy for detection of IgG antigen by combining the rolling circle amplification (RCA) technique with oligonucleotide functionalized carbon dots (CDs), based on a paper-based electrochemiluminescence (ECL) origami device (PECLOD) for the first time. In this PECLOD, three-dimensional (3D) macroporous Au-paper electrode was fabricated and employed as the working electrode for specific and efficient antibodies capture. The RCA product containing tandem-repeat sequences could serve as an excellent template for periodic assembly of CDs, which presented per protein recognition event to numerous CDs tags for ECL readout. Under the optimal conditions, the proposed strategy showed remarkable amplification efficiency, very little nonspecific adsorption with good stability, reproducibility, and accuracy. Using human IgG (H-IgG) as a model protein, the designed strategy was successfully demonstrated for the ultrasensitive detection of protein target. The results revealed that the strategy exhibited a dynamic response to H-IgG range from 1.0 fM to 25 pM with a limit of detection as low as 0.15 fM. Importantly, the methodology can be further extended to the detection of other proteins or biomarkers.

1. Introduction

Paper-based materials continue to be harnessed in a growing number of applications, motivated, in part, by a low-cost, high strength-to-weight ratio, and inherent resource renewability since the first production from cellulosic plant fibers centuries ago. While the use of paper for biological assays and point-of-care diagnostics is not a new concept (Comer, 1956), it is still attracting much more attention especially after microfluidic paper-based analytical devices (μPADs) were firstly reported by Whitesides' group (Martinez et al., 2007, 2008) as cheap platforms for lab-on-paper applications. The most obvious benefits are the low cost of paper and the highly developed infrastructure of the printing industry, making production of μPADs both economical and scalable. Our group has developed a series of analytical applications on μPADs for the sensitive detection of small molecules, proteins, and DNAs (Ge et al., 2013a, 2013b; Zhang et al., 2014). The usage of μPADs provides new opportunities and directions in the development of precise and sensitive diagnostic devices for biological samples.

An ultrasensitive and feasible method for detecting and quantifying biomarkers is important in biological studies, clinical diagnostics, and treatment (Wang et al., 2011; Wu et al., 2003; Hou et al., 2014; Shi et al., 2013). In general, immunoassay-based protocol employing antibody–antigen interactions is a versatile and powerful analytical technique in the quantitative detection of biomarkers due to the highly specific molecular recognition of immunoreaction (Chikkaveeraiah et al., 2012; Kimmel et al., 2012). Typically, the assay is performed using certain affinity ligands comprising aptamers and antibodies that specifically interact with the biomolecules and thus mediate a target-responsive signal transduction cascade (Tabkman et al., 2011; Nie et al., 2009). Recently, great efforts have been made to develop new detection techniques for biomarkers to improve sensitivity (Schweitzer et al., 2000; Mani et al., 2009; Fan et al., 2005; Fu et al., 2007; Li et al., 2008). Among the diverse techniques, the electrochemiluminescence (ECL), integrating the merits of chemiluminescence and electrochemistry, such as low cost, wide range of analytes, low background signal, and high sensitivity, has also been extensively used for biosensing (Duan et al., 2010; Xu et al., 2011). Especially when established on μPADs (Delaney et al., 2011),

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ECL not only continues to impact diverse areas ranging from chemical analysis to the molecular-level understanding of biological processes, but also increases the scope of options for detections on μPADs.

As biomarker detection still been a major challenge, DNA amplification techniques based on polymerase chain reaction (PCR) have been explored as important strategies for sensitive protein assays (Zhang et al., 2007). Different assay formats such as liposome-PCR (Mason et al., 2006), phage display mediated immuno-PCR (Guo et al., 2006), and aptamer-based affinity PCR (Zhang et al., 2006) have been developed to increase the abundance of the detection probes and significantly lower the limit of detection for protein analysis. Although these assays in principle offer extremely high sensitivity and wide quantitative dynamic range, they require thermal cycling and strict laboratory conditions to avoid contamination or false results, and are usually too complicated and time-consuming for practical applications. Rolling circle amplification (RCA), an isothermal DNA amplification procedure (Fire and Xu, 1995), can generate a linear concatenated DNA molecule containing up to 1000 complementary copies of the circular DNA in 1 h (Baner et al., 1998). Compared with PCR, this technique can simplify operation steps and save time due to the isothermal amplification procedure and the linear kinetic model (Zhou et al., 2007).

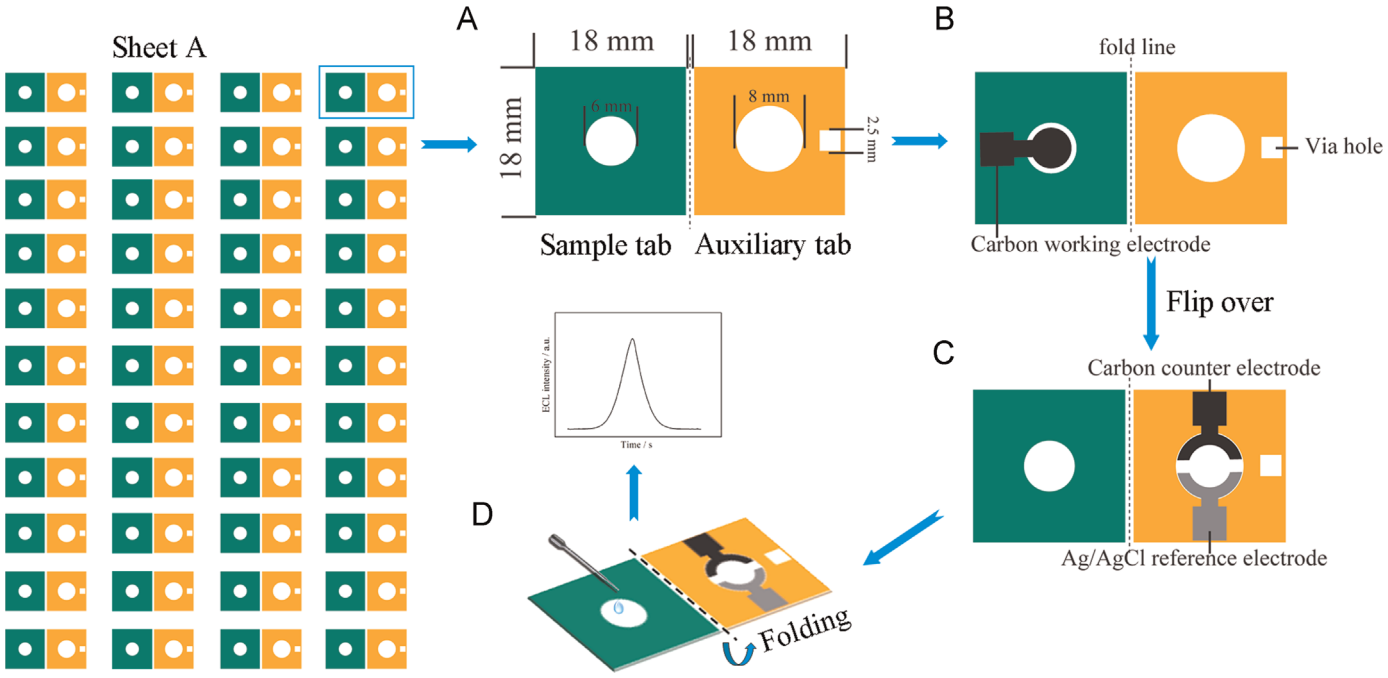
Herein, we first report the proof-of-concept of a novel and powerful immuno-RCA assay strategy for determination of larger target analytes, e.g., proteins, by coupling the amplification capability of the RCA with the sensitive ECL signal of CDs molecules conjugated to probes in a paper-based ECL origami device (PECLOD) (Scheme 1). The human IgG (H-IgG) was used as a model protein to verify the practicability of the proposed strategy in this work. As shown in Scheme 2, after the secondary antibodies on the gold nanoparticles conjugated with primer strands (Ab_2–AuNPs-primer) which are single-strand oligonucleotides to act as RCA primers for binding of the circular template, the RCA was initiated to produce micrometer-long single-strand DNA in the presence of nucleotides and phi 29 DNA polymerase. The products of the RCA reaction contained hundreds of tandem-repeat sequences for linear periodic assembly of a large number of DNA complementary detection probes which could amplify the detection signals significantly. ECL intensity increase of the cascade DNA nanotags further enhanced the sensitivity of the proposed protocol for monitoring the recognition events of the target protein.

2. Experiments

2.1. Materials and reagents

The oligonucleotides with the following sequences were obtained from the Shanghai Linc-Bio Science Co. Ltd. (Shanghai, China). Primer, 5′-SH-$(CH_2)_6$ -AAA AAA AAA AAA AAA CAC AGC TGA GGA TAG GAC AT-3′; circular template, 5′-p-CTC AGC TGT GTA ACA ACA TGA AGA TTG TAG GTC AGA ACT CAC CTG TTA GAA ACT GTG AAG ATC GCT TAT TAT GTC CTA TC-3′; DNA probe, 5′-NH_2-TCA GAA CTC ACC TGT TAG-3′. Phi 29 DNA polymerase, T4 DNA ligase, and dNTP were obtained from Fermentas (Lithuania). Salmon sperm DNA, streptavidin, N-hydroxysuccinimide (NHS) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (USA). Human immunoglobulin G (H-IgG), capture antibodies of IgG (Ab_1) and monoclonal antihuman IgG (Ab_2) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). 1-Ethyl -3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was purchased from Pierce (Rockford, IL).

The physiological buffer saline (PBS) consisted of 0.15 M NaCl, 7.6 mM NaH_2PO_4, and 2.4 mM Na_2HPO_4 (pH 7.4). PBS-T buffer consisted of 0.15 M NaCl, 7.6 mM Na_2HPO_4, 2.4 mM Na_2PO_4, and 0.05% Tween-20 (pH 7.4). Ultrapure water obtained from a Millipore water purification system (resistivity ≥ 18 MΩ cm) was used in all assays and solutions. Whatman chromatography paper #1 (200.0 mm × 200.0 mm) (pure cellulose paper) was obtained from GE Healthcare Worldwide (Pudong Shanghai, China) and used with further adjustment of size. The Ag/AgCl ink (CNC-01) and carbon ink (ED-581-SS) for screen-printed electrodes were



Scheme 1. Schematic representation of the fabrication and assay procedure of this origami device. Paper sheets were first patterned in bulk using a wax printer (sheet A). (A) One 3D origami device without the screen-printed electrodes. (B, C) Then electrodes were screen-printed on sheet A in bulk. Finally, sheet A was cut into individual 3D origami device. (D) After modification, the device was used to detect IgG.