Biosensors and Bioelectronics 71 (2015) 18–24

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Self-powered competitive immunosensor driven by biofuel cell based on hollow-channel paper analytical devices

Shuai Li [a], Yanhu Wang [a], Shenguang Ge [b], Jinghua Yu [a,*], Mei Yan [a]

[a] Key Laboratory of Chemical Sensing & Analysis in Universities of Shandong, School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, PR China

[b] Shandong Provincial Key Laboratory of Preparation and Measurement of Building Materials, School of Material Science and Engineering, University of Jinan, Jinan 250022, PR China

ARTICLE INFO

Article history:
Received 16 January 2015
Received in revised form
5 April 2015
Accepted 6 April 2015
Available online 7 April 2015

Keywords:
Biofuel cell
Microfluidic paper-based analytical devices
Glucose dehydrogenase
Nanoporous PtNi
Bilirubin oxidase

ABSTRACT

A mediator-less and compartment-less glucose/O_2 enzymatic biofuel cell (BFC) was introduced into microfluidic paper-based analytical devices (μ-PADs) that relies on flow in hollow channels with silver nanoparticles/graphene modified paper electrode as the anodic and cathodic substrate, to implement self-powered sensitive carcinoembryonic antigen (CEA) detection. Glucose dehydrogenase (GDH)–gold nanoparticles bioconjugate modified with CEA acted as a biocatalyst for enhancing glucose oxidation in the bioanode, as well as the transducing enzyme for signaling magnification. Similarly, nanoporous PtNi/bilirubin oxidase (BOD) acted as a biocatalyst for enhancing O_2 reduction in the biocathode. With an increase in the concentration of CEA, the amount of CEA–Au–GDH bioconjugate on bioanode decreases, thus leading to the lower output of the as-prepared BFC. This proposed BFC-based self-powered immunosensor for CEA possessed largely increased linear detection range from 1 pg mL^{-1} to 0.5 μg mL^{-1} with a detection limit of 0.7 pg mL^{-1}. The proposed BFC-based self-powered immunosensor shows high sensitivity, stability, and reproducibility and can become a promising platform for other protein detection.

1. Introduction

In clinical analysis, increased levels of tumor markers in human serum are associated with patients with cancer, one of the most threatening diseases for human beings (Song et al., 2010). They also offer opportunities for understanding the fundamental biological processes involved in disease progression and monitoring patient responses to therapy methods, which can promote the understanding of cancer diseases related biological processes (Xu et al., 2011; Zhang et al., 2013a). Therefore, sensitive and accurate assays for tumor markers in complex biological samples will be valuable for disease screening and diagnosis. Conventional immunoassays for the detection of biomarkers include enzyme-linked immunosorbent assay (ELISA) (Brochot and Siddiqi, 1989), electrochemistry (Liu et al., 2010), electrochemiluminescence (ECL) (Cao et al., 2011), mass spectrometry (Hu et al., 2007), quartz crystal microbalance (QCM) (Chou et al., 2002), and surface plasmon resonance (SPR) immunoassays (Teramura and Iwata, 2007). While highly accurate, some of these techniques involve disadvantages such as relatively sophisticated instruments, significant sample volume, limited sensitivity, and clinically unrealistic expense and time. Thus it is urgent to develop immunoassays with easy-to-use, fast, inexpensive, highly sensitive, miniaturized analytical devices and on-site monitoring. As a new kind of green energy conversion technology, biofuel cell (BFC) is drawing increased attention which extract bioenergy from biochemical reactions to produce electricity and exhibit many advantages such as the capability for detection without external power sources, simplifying the fabrication process, minimizing the scale, and reducing the expense, which is especially beneficial to the miniaturization of detection devices (Zhang et al., 2012; Miyake et al., 2011). Herein, a new concept of a BFC-based self-powered immunosensor was developed for CEA detection.

Microfluidic paper-based analytical devices (μ-PADs) which are particularly well-adopted as a platform for the development of simple and cost-effective molecular diagnostic assays have been drawn increasing interest since Martinez et al. (2007) pioneered this field from 2007. As an alternative to the first-generation lab-on-chip devices, the past six years have witnessed fast development and great research efforts in the field of μ-PADs with focuses on developing novel fabrication techniques (Martinez et al., 2010;

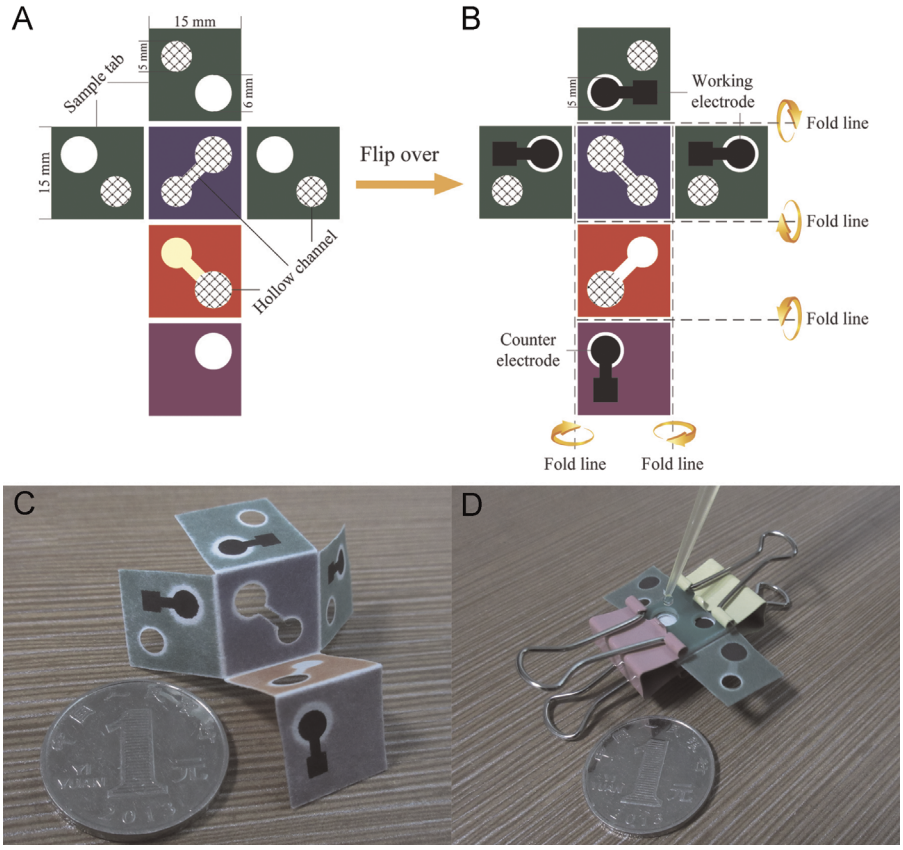
* Corresponding author.
E-mail address: ujn.yujh@gmail.com (J. Yu).

http://dx.doi.org/10.1016/j.bios.2015.04.019
0956-5663/

Ballerini et al., 2012), inventing new functional concepts (Liu et al., 2013; Li et al., 2013), and designing new prototype μ-PADs for analytical applications (Zhang et al., 2013; Lewis et al., 2012). To date, various scientific and technical about analytical methods on μ-PADs have been demonstrated (Carrilho et al., 2009; Delaney et al., 2011). However, some limitations of the slow fluids flow in paper channels were also distinct, such as time-consuming and solvent evaporation which are not beneficial to point-of-care testing. Recently, instead of cellulose network, μ-PADs with hollow channels for fluids transport have been demonstrated (Renault et al., 2013). Compared with cellulose networks, the hollow channel μ-PADs possessed the distinguished advantage of easy fabrication, with no pump requirement and rapid fluid flow facilitation appropriate for point-of-care testing (Renault et al., 2014). Inspired by this simple technique, a self-powered immunosensor based on glucose/O_2 BFC device was introduced into the μ-PADs with hollow-channel in this work. For combining the BFC device with μ-PADs, a 3D microfluidic hollow-channel origami-based BFC analytical device (3D-μ-HCOBFCAD) was demonstrated based on origami principle using wax-patented technology, screen printed and laser cut.

Rational design of electrode with excellent performance for magnifying the sensitivity is highly desirable for achieving better BFC-based self-powered immunosensors. Various nanostructures have been introduced as electrode enhancing materials to immobilize biocatalysts and therefore improve the performance of BFC (Zebda et al., 2011; Wen et al., 2011; Pan et al., 2010). Graphene (G), a single layer of carbon atoms in a closely packed honeycomb two-dimensional lattice, has been attracted enormous interests in constructing electrochemical biosensors due to its novel properties such as high thermal and chemical stability, large specific surface area and outstanding conductivity (Dong et al., 2012; Wei et al., 2010; Li et al., 2014). To further increase the surface area, biocompatibility, as well as improving the electronic transmission rate, silver nanoparticles-functionalized graphene (Ag–G) platform was established through a photo and chemical reduction method.

In this work, we described a BFC-based self-powered immunosensor integrated with hollow channel based μ-PADs for CEA detection using Ag–G nanocomposite modified paper electrode as the substrate for both bioanode and biocathode. A competitive reaction based on the specific binding of anti-CEA antibodies to CEA and CEA–Au–GDH bioconjugates was achieved in bioanode. Meanwhile, nanoporous PtNi alloy–bilirubin oxidase (BOD) nanocomposites showed direct bioelectrocatalytic function for the reduction of O_2 to H_2O at biocathode (Wen et al., 2014). When the bioanode was exposed to a solution containing glucose, the GDH can catalyze the conversion of glucose into gluconolactone, protons and electrons. Meanwhile, BOD encapsulated into nanoporous PtNi realized the direct electrocatalytic reduction of O_2 with synergetic effect. Under optimal conditions, the proposed BFC-based self-powered immunosensor showed wide detection range and low detection limit. Additionally, the results demonstrated that the developed 3D-μ-HCOBFCAD supplied a convenient, low-cost, and sensitive method for tumor markers detection. Moreover, this strategy offers a step toward the goal of conducting quantitative point-of-care assays without using auxiliary instruments or electronics.



Scheme 1. The schematic representation, size, and shape of this 3D-μ-HCOBFCAD. (A) One side of the 3D-μ-HCOBFCAD without the screen-printed electrodes. (B) The reverse side of (A) with the screen-printed electrodes. (C) The schematic of this folding 3D-μ-HCOBFCAD. (D) The schematic of the assay procedures. (For interpretation of the references to color in this scheme, the reader is referred to the web version of this article.)