Sensing and Bio-Sensing Research 20 (2018) 41–46

Contents lists available at ScienceDirect

Sensing and Bio-Sensing Research

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

Sensitive electrochemical detection of glucose via a hybrid self-powered biosensing system

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ARTICLE INFO

Keywords:
Biofuel cell
Energy harvesting
Bilirubin oxidase
Self-powered glucose biosensing
Electrochemical sensor

ABSTRACT

The importance of glucose in many biological processes continues to garner increasing research interest in the design and development of efficient biotechnology for the sensitive and selective monitoring of glucose. Here we report on a novel hybrid self-powered biosensing system with a unique capability to convert the biochemical energy of glucose into electrical power, which is subsequently stored in a 10 pF capacitor serving as the transducing element. The anode and biocathode of the hybrid cell were constructed from a gold-supported nanoporous colloidal platinum structure (Au-co-Pt) and bilirubin oxidase (BOD) modified gold coated Buckypaper (BP-Au-BOD), respectively. The hybrid cell delivered an open circuit voltage and short circuit current of 0.73 V and 0.50 mA, which was ample to drive an energy amplification circuit and generate sufficient power to power an LED via the 10 pF capacitor. The self-powered glucose biosensing system exhibited excellent electrocatalytic activity towards glucose oxidation with a linear dynamic range up to 18 mM glucose. The biosensor demonstrated excellent selectivity towards glucose in the presence of interfering species. This presented hybrid self-powered biosensing system holds great promise to develop a self-contained continuous monitoring systems for a variety of biomedicine applications.

1. Introduction

The disease diabetes mellitus is one of the leading causes of death and disability in the world. This metabolic disorder results from insulin deficiency, which is reflected by blood glucose concentrations higher or lower than the normal range of 80–120 mg/dL (4.4–6.6 mM). As of 2015, an estimated 415 million people had diabetes worldwide, which continues to grow according to the recent World Health Organization (WHO) report in 2018 [1]. A sedentary lifestyle combined with changes in eating habits has resulted in significant increases in obesity. In addition, the prevalence of obesity has been attributed to the major causes of the increased rates in individuals diagnose with diabetes [2]. The major complications associated with the disease diabetes are high risks of heart disease, kidney failure, and/or blindness. These complications can be greatly reduced through stringent control of blood glucose by continuous monitoring of blood glucose to provide data for optimizing and/or changing patient treatment management strategies.

Accordingly, millions of individual living with diabetes test their blood glucose levels daily, thereby making glucose the most commonly tested analyte. Glucose biosensors or glucometers account for about 85% of the entire biosensor market [3]. Since the initial concept of glucose enzyme electrodes was proposed in early 1960's, considerable amount of fascinating research and innovative detection strategies have been developed in the field of glucose biosensing because of the tremendous economic prospects associated with diabetes and the challenge of providing reliable and accurate measurement of blood glucose.

Electrochemical sensing, by virtue of its rapid response time, ease of use, low-cost, portability, reliability, sensitivity, and selectivity, is one of the promising and widely accepted technique for glucose biosensing [4–7]. The three major components of an electrochemical biosensor are the recognition element, electrochemical transducer and a signal processing unit for the recording, signal amplification and user-friendly data representation [8–11]. Moreover, the recognition element is the primary component of a biosensor, which allows the sensor to respond selectively to one particular analyte from among a large number of other analytes. Depending on the type of recognition element, biosensors can be classified as enzymatic, non-enzymatic, whole-cell and immunosensors [12, 13]. Moreover, electrochemical biosensors based on nanomaterials have recently attracted considerable attention due to their unique chemical and physical properties [14, 15], wherein gold nanoparticles (AuNPs) [16], carbon nanotubes [17], metallic oxides [18], and semiconductors [19] have been used widely in the fabrication of biosensors for medical analysis, environmental monitoring, and food quality control. The unique properties of gold nanoparticles have been

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https://doi.org/10.1016/j.sbsr.2018.08.002
Received 22 June 2018; Received in revised form 1 August 2018; Accepted 1 August 2018

shown to provide a suitable microenvironment for biomolecule immobilization and to facilitate electron transfer between the immobilized protein and the electrode substrate. This has led to an intensive use of those nanomaterials for the construction of electrochemical biosensors with enhanced analytical performance with respect to other biosensor designs [20, 21]. Despite the advances made in the design and development of glucose biosensors, the promise of the development of self-contained and self-powered biosensors to enable tight blood glucose management has not been fulfilled.

Therefore, significant research efforts have been made to develop enzymatic-based biofuel cells, which generate bioelectricity via oxidation-reduction (redox) reactions between the biofuel and the oxidant to power miniaturized glucose biosensors. The most commonly used enzymes in glucose based biofuel cells are glucose selective enzymes (i.e., glucose oxidase and glucose dehydrogenase) and oxygen selective enzymes (i.e., laccase and bilirubin oxidase). Enzymatic glucose biofuel cell typically employ laccase as the oxygen reducing enzyme due to its great affinity towards oxygen reduction. However, its optimal operating pH range is limited to 5.5–6 [22], which is well below the physiological pH. In order to implement enzymatic glucose biofuels at physiological pH, the laccase biocathode in the enzymatic glucose biofuel cell would prove to be ineffective. Bilirubin oxidase has been demonstrated as an ideal oxygen reducing enzyme in glucose based biofuel cells that can achieve higher power density at physiological pH [22–24]. However, the main limitation of these enzymatic biofuel cell lies in the limited low power densities generated and the inactivity of the enzymes overtime.

In the present work, a 3-strand braided Au microwire electrode was constructed and employed as the substrate for the electrodeposition of colloidal platinum nanostructures (Au-co-Pt), which was accomplished using a platinizing solution. This abiotic anode enables the direct oxidation of glucose. The enzyme based cathodic substrate comprise a mesh dense network of multi-walled carbon nanotubes (MWCNTs) coated with a thin layer of Au followed by covalent immobilization of BOD (BP-Au-BOD) via 3-mercaptopropionic acid self-assembled monolayers and subsequent EDC/s-NHS (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride and N-Hydroxysulfosuccinimide) coupling. The abiotic anode and enzymatic cathode were assembled and integrated with an energy amplification circuit and a capacitor functioning as a glucose transducer to construct the hybrid self-powered electrochemical glucose biosensor.

2. Experimental section

Buckypaper, a compressed network of multi-walled carbon nanotubes (MWCNTs) was purchased from NanotechLabs (Yadkinville, NC). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) was purchased from Pierce Chemicals. Au microwires ($\phi = 250\,\mu m$), glucose, uric acid, isopropyl alcohol (IPA), ethanol, ascorbic acid, acetaminophen, sodium hydroxide, mercaptopropionic acid (MPA), potassium phosphate monobasic, glycol-chitosan and bilirubin oxidase (BOD) were acquired from Sigma-Aldrich (St. Louis, MO, USA). The platinizing solution was purchased from YSI Inc. (Yellow Springs, USA). Phosphate buffer saline solution pH 7.4 were prepared with 18.2 MΩ cm Milli-Q water.

To prepare the abiotic anode to exhibit high electrocatalytic surface area for the oxidation of glucose, 3 strands of 7 cm gold microwires were braided as shown in Fig. 1. The electrode was cleaned with IPA for 5 min and dried with nitrogen gas. The cleaned electrode was immersed in platinizing solution and using electrochemical deposition method, which was based on a three-electrode system with the Au braided microwire, Pt wire, and Ag/AgCl electrodes serving as the working electrode, counter electrode and reference electrode, respectively. Colloidal Pt was electrodepositied from platinizing solution at an applied potential of -225 mV vs. Ag/AgCl for 1500 s. The prepared anode was then rinsed in deionized water to remove any loosely bound platinum particles and dried at 260 °C for 5 min, followed by cooling in ambient air.

The biocathode was prepared by sputtering a thin film of gold (~ 40 nm) on buckypaper to result in BP-Au electrode, which was then cut into 5 mm × 34 mm sections and cleaned with IPA for 5 min. This BP-Au electrode was used as the backing material for the immobilization of BOD. The BP-Au electrode was treated with 1 mM MPA for 5 h to form self-assembled monolayers with the thiol (-SH) tail groups of MPA establishing a strong covalent bond with the BP-Au surface and the carboxylic acid functional head group exposed for activation [25]. The MPA treated BP-Au electrode was washed with 10 mM PBS solution to remove any unbound MPA. The carboxylic acid functional groups were then activated via EDC/s-NHS (2:1, MES buffer pH 4.7) and 1.0 mg of BOD in 0.1 M PBS 7.4 for 3 h. In presence of the EDC, the N-hydroxyl group of the s-NHS interacts with the carboxyl groups to form reactive sites, wherein the amino groups of BOD displace the succinimide group of s-NHS to form covalent amide bond between the carboxylic and the amino functional groups. The prepared BP-Au-BOD electrode was washed with deionized water to remove any unbound BOD. The BOD treated BP-Au was then coated with 1 wt% chitosan and dried under ambient conditions. The BP-Au-BOD biocathode was stored in 0.1 M PBS solution (pH 7.4) at 4 °C when not in use. The electrochemical measurements were performed using a BASi-Epsilon electrochemical workstation (BASi, West Lafayette, IN, USA) using a three electrode configuration at room temperature.

3. Results and discussion

The morphology of the Au-co-Pt abiotic anode is illustrated by scanning electron microscopy (SEM) in Fig. 1. Prior to electrodeposition of platinum, the clean gold microwire possessed a smooth surface as shown in Fig. 1A. Following the electrodeposition, most of the colloidal Pt electrodeposited on the braided Au (Fig. 1B-1C) and are observed to form uniform flowerets of colloidal platinum with an average size of 7.5 ± 1.9 μm. The high resolution SEM image of the Au-co-Pt (Fig. 1B) shows that they have porous nanostructures with high surface-to-volume ratio, which really seem like they are assembled from hundreds of nanocomposites.

For comparison, the high magnification SEM image of the gold coated buckypaper (BP) shows that the BP exhibits a porous, interconnected, 3D dense mesh network of MWCNTs (Fig. 2A). After the covalent immobilization of BOD, the porous 3D dense mesh network of BP-Au-BOD (Fig. 2B) SEM revealed ultrafine netting structures with a thick coating of BOD covalently bound to the surface. This was not observed in the SEM images of the BP-Au samples (Fig. 2A). According to the analyses of randomly-selected nanowires, the thickness of the MWCNTs before and after BOD immobilization were 28 ± 8 nm and 38 ± 5 nm, respectively. Here BOD was covalently-attached on the mercaptopropionic acid (MPA) treated gold coated MWCNTs by employing the EDC/s-NHS coupling chemistry to conjugate the carboxyl groups on BP-Au-MPA with the amino groups on BOD.

We investigate the electrochemical performance of the biocathode to determine whether an electronic connection was established between the BOD and MWCNT. The activity of immobilized BOD was measured by the electrochemical reduction of molecular oxygen to produce water. Cyclic voltammograms (CVs) were used to evaluate the electrochemical activity of the immobilized BOD via MPA functionalization. Fig. 3 shows the CVs of BP-Au-BOD biocathode recorded in 0.1 M PBS solution (pH 7.4) in the absence and presence of saturated oxygen at a scan rate of 20 mV s^{-1}. Only a small evidence of electrocatalytic activity for oxygen reduction was observed in the absence of saturated oxygen (Fig. 3, curve a). In the presence of saturated oxygen, the CV results depicted strong evidence of electrocatalytic activity for oxygen reduction (Fig. 3, curve b). The reserve scan depicted a dramatic deflection from the biocathode in the absence of saturated oxygen with an onset potential of 400 mV. This enhanced electrochemical activity of