Biosensors and Bioelectronics 24 (2009) 3419–3424

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

A micro-potentiometric hemoglobin immunosensor based on electropolymerized polypyrrole–gold nanoparticles composite☆

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ARTICLE INFO

Article history:
Received 14 April 2008
Received in revised form 25 July 2008
Accepted 29 July 2008
Available online 15 August 2008

Keywords:
Micro-immunosensor
Potentiometric
Polypyrrole (PPy)
Gold nanoparticles (AuNP)
Hemoglobin (Hb)
Ion-sensitive field-effect transistors (ISFETs)

ABSTRACT

We report a novel micro-potentiometric hemoglobin (Hb) immunosensor based on electrochemically synthesized polypyrrole (PPy)–gold nanoparticles (AuNPs) composite. PPy–AuNPs film with AuNPs uniformly distributed in it was deposited on gold electrode surface by a simple and direct procedure, without the addition of any nanoparticles or reducing agent. And this generic method makes it possible to deposite different polymers on miniaturized electrodes. With the existence of AuNPs, the antibody immobilization onto the electrode surface was facilitated. Morphology study by field emission scanning electron microscope (FE-SEM) confirms the presence of AuNPs in PPy. Based on an ion-sensitive field-effect transistors (ISFETs) integrated chip, a micro-potentiometric immunosensor for Hb and hemoglobin-A1c (HbA1c) has been constructed. The sensor response was linear over the concentration range 60–180 μg/ml Hb and 4–18 μg/ml HbA1c. The Hb concentration in whole blood samples has also been analysed, with a linear dose–response behavior between 125 and 197 μg/ml and a sensitivity of 0.20 mV μg^{-1} ml. The measuring ranges of the developed Hb and HbA1c immunosensors meet the clinical demand for measuring the HbA1c/Hb ratio of 5–20%. This sensor results in simple and rapid differential measurement of Hb and HbA1c, and has great potential to become an inexpensive and portable device for monitoring of diabetes.

1. Introduction

Hemoglobin-A1c (HbA1c) is a kind of minor hemoglobin formed by a non-enzymatic reaction of glucose with the amino-terminal valine of the hemoglobin (Hb) β-chain (Bunn et al., 1976). The HbA1c level reflects the blood glucose concentration of the previous 2–3 months. Therefore, clinical methods for the determination of HbA1c has become an established procedure in the diagnosis and monitoring of diabetes (McDonald and Davis, 1979). HbA1c is measured as a relative content of total Hb with the clinical range 5–20%, and 4–6% is estimated as the normal value for a healthy adult. Immunosensors for HbA1c and Hb measurement have been reported, including amperometric immunosensors (Liu et al., 2006; Stöllner et al., 2002), capacitance-based immunosensors (Haysa et al., 2006), piezoelectric sensors (Halámek et al., 2007; Pribyl and Skládal, 2005), etc. However, the existing amperometric HbA1c immunosensors are relatively time-consuming because the antibody/antigen-labeling reaction is involved. Most of the other existing methods require dedicated equipment and therefore they can only be operated at laboratories by trained staff. A miniaturized device which can carry out inexpensive and portable HbA1c and Hb analysis is highly desired (Cagliero et al., 1999).

Recently, biosensors based on microelectronics and MEMS techniques have received considerable attention, such as ion-sensitive field-effect transistors (ISFETs). ISFET biosensors can achieve rapid and label-free detection of a wide range of chemical and biological species. Further more, since ISFET biosensors can be fabricated by the standard CMOS process, they could be reduced in size and mass-produced. They are known as one of the most important miniaturized biosensors (Liu et al., 2007). Micro-potentiometric HbA1c and Hb immunosensors based on ion-sensitive field-effect transistors have great potential to become a portable device and help for glycemic monitoring of diabetic patients.

For potentiometric immunosensors, the general preparation procedure involves the immobilization of immunoactive entities (antibodies and the like) onto electrochemical transducer surfaces. With the electrode miniaturization, the technique of depositing different biocompatible films on miniaturized electrodes and the easy control of film characteristics are of great importance. The electrochemical polymerization of conducting polymers such as polypyrrole (PPy) has been considered a useful method for immunosensing application, due to their high chemical stability, compatibility with immunoactive entities and facility to be doped

☆ This article is a part of the special issue "Biosensors 2008".

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doi:10.1016/j.bios.2008.07.077

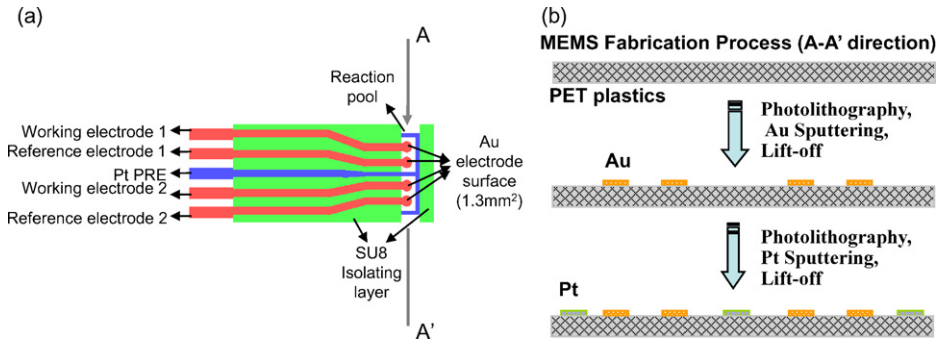

Fig. 1. Schematic diagrams of (a) the electrode chip of the immunosensor and (b) the MEMS fabrication process of the electrode.

with nanoparticles (Adeloju and Moline, 2001; Razola et al., 2002; Kum et al., 2007; Wu et al., 2007; Li et al., 2005).

However, there still exists challenges in the design of label-free micro-immunosensors, especially the diminished sensitivity coming with the process of electrode miniaturization (Wang et al., 2005). To overcome this problem, several strategies are employed to enhance antibodies immobilization, including the incorporation of nanoparticles (Chu et al., 2007; Li and Lin, 2007; Viticoli et al., 2006; Du et al., 2007; Chen et al., 2007), the use of porous materials to enlarge effective electrode surface area (Liu et al., 2006; Mala Ekanayake et al., 2007), and the orientation-controlled antibodies immobilization techniques (Wang et al., 2004). Recently, gold nanoparticles (AuNPs) have raised growing interest due to their unique size-dependant properties. They have been intensively studied in bioreagents immobilization via the large specific interface area, desirable biocompatibility and high surface free energy of nanosized particles (Riu et al., 2006; Guo and Wang, 2007). It was found that the conductivity and the sensing behavior of conductive polymers could be further improved by imbedding metal particles into the polymer matrix to form a metal–polymer composite (Strike et al., 1992; Rau et al., 1994). Enzyme biosensors based on electrodes with nanostructured PPy–gold composite have been reported (Njagi and Andreescu, 2007). But the existing methods for synthesis of polymer–gold nanoparticles composite are time-consuming, and the addition of gold nanoparticles or reducing agent is usually required. And these methods are not suitable for the selective growth of the composite film on miniaturized electrodes. Moreover, the congregation of AuNPs in polymer film remains an unsolved problem.

Here we present a simple and direct procedure for the formation of PPy–AuNPs composite film, with the electrochemical growth of AuNPs in PPy. Based on this novel method, the antibody immobilization onto micro-gold electrodes was enhanced, and a micro-potentiometric Hb/HbA1c immunosensor based on ISFETs has been constructed and studied. Field emission scanning electron microscope (FE-SEM) and cyclic voltammetry (CV) were conducted to characterize the immunosensor. Immunosensor performances were tested by the measurement of Hb, HbA1c and whole blood samples.

2. Experimental

2.1. Sensor design

The micro-potentiometric immunosensor consists of a MEMS fabricated micro-electrode and an integrated ISFET chip. The ISFET chip contains two ISFETs, two reference FETs (REFET) and the signal read-out circuits. The integrated ISFET chip containing two n-channel FETs were fabricated using a commercial 0.35 μm, 4-metal and 2-poly CMOS process (MPW, Chartered Semiconductor, Singapore). In the experiments ISFETs with a channel length of 2 μm were used. The manufacturing process has been described elsewhere (Wei et al., 2006). Two gold working electrodes, two gold reference electrodes and a platinum pseudo-reference electrode (PRE) are designed to construct the micro-electrode, as shown in Fig. 1a.

After packaging, the ISFET chip was mounted on a standard printed circuit board. The bonding pads of the chip were connected to the micro-electrode, the power source and a data acquisition card. The working electrodes and the reference electrodes were connected to the gates of the FETs, working as floating gate of FET. The working electrodes are connected to ISFET, and the reference electrodes are connected to REFET.

PPy–AuNPs composite and pure PPy were respectively deposited on the working electrodes and the reference electrodes. Immobilization of immunoactive entities and inactivated entities are carried out on the working electrode and the reference electrode respectively, so as to achieve differential measurement. Based on immobilization of Hb antibody or HbA1c antibody on the PPy–AuNPs, a hemoglobin/hemoglobin-A1c immunosensor was constructed. The PRE is used as the reference electrode and counter electrode during the electrode preparation and potentiometric measurement. An SU-8 micro-reaction pool is designed to enclose the reaction solutions.

The antibody immobilization strategy employs the electrochemical synthesis of PPy–gold chloride acid ($HAuCl_4$) composite on gold electrode surface, the electrochemical reduction of $HAuCl_4$ into AuNPs, and the adsorption of antibodies to this composite film, as illustrated in Fig. 2.

2.2. Reagents and apparatus

Hb and HbA1c antibody was supplied by Beijing Yicheng Bioelectronics Technology Co., Ltd. (Beijing, China). Hb and HbA1c was purchased from Fitzgerald. Gold(III) chloride trihydrate acid ($HAuCl_4{\cdot}3H_2O$), bovine serum albumin (BSA) and phosphate buffered saline (PBS, pH 7.4) tablets were purchased from Sigma–Aldrich. Pyrrole was from Fluka. Tiron, Na_2SO_4, etc. were obtained from Beijing Chemical Reagent Company (China). All chemicals used were of analytical grade. Deionized water was used throughout the experiments.

Cyclic voltammetric measurements and electrochemical synthesis of PPy film and AuNPs were carried out with a Microcomputer Electrochemical Analyzer RST 3100 (Suzhou Risetech Instrument Co., Ltd., China). SEM analysis was carried out to determine the morphological structures of the modified gold electrode using an S-4800 field emission scanning electron microscope (FE-SEM) produced by Hitachi (Japan).