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An enzyme–metal–insulator–silicon structured sensor using surface photovoltage technology for potentiometric glucose detection

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ABSTRACT

In this study a potentiometric glucose sensor is constructed with the application of an enzyme–metal–insulator–silicon (EMIS) structure. Glucose biosensing is realized by modifying the metal layer of the sensor with an ultra-thin (<100 nm) film of polypyrrole (PPy)–glucose oxidase (GOD) through an electropolymerization process. The optimum film formation conditions can be provided with 0.1 M pyrrole, 100–200 U/mL GOD, an applied current density of 0.01–0.05 mA/cm^2 and an electrical charge of 20–30 mC/cm^2. The applicability of the surface photovoltage technology for potential determination is confirmed with an improved sensitivity (106.3 mV/dec) and widened linear range (0.04–10 mM) compared with the traditional two-electrode cell measurement. Good selectivity, stability and lifetime of the potentiometric glucose sensor are also shown. The usage of the ultra-thin PPy–GOD film is advantageous in reducing the response time (from several seconds to less than 80 s) of the sensor, which guarantees its potential in rapid determination of plasma glucose concentration. With ease of fabrication and miniaturization, the photoelectric hybrid glucose sensor can be used in glucose monitoring of extracellular microenvironment.

1. Introduction

Since glucose works as the main energy source of living cells, it is significant that the glucose concentration maintains in a normal range (usually 85–135 mg/dL) to keep the physiological state for many kinds of cells. The detection of glucose has attracted most interest since the first publication on a glucose sensor [1]. There has been a constant increase in the number of studies devoted to glucose biosensing [2], among which amperometric technique is most widely used [3–7]. Although the amperometric glucose sensor has relatively high sensitivity and low detection limit, it seems to exhibit some disadvantages such as selectivity due to the interference of other reducible species in plasma. Even the application of nanostructured metal-oxides in the state-of-the-art research activities cannot generate satisfactory sensitivity, selectivity and stability simultaneously [8,9]. Besides, the electrocatalytic oxidation is not conducive to the continuous monitoring of glucose in vivo. In contrast, the potentiometric glucose detection [10–13] would be subject to fewer interferences, and it is independent of the size of the indicating electrode. In this connection, the potentiometric method would have great potential applications in long-time and continuous glucose monitoring.

Potentiometry is commonly used to measure glucose concentrations greater than 10^{-5} M, which is in the physiological range in most cases. So far the potentiometric glucose sensors are mostly enzyme-based. The response time and the stability of these sensors are varied with different GOD immobilization methods. Conducting polymers, such as polypyrrole (PPy) [14–18], have been widely used for enzyme immobilization [19,20] over the last two decades because of their capability of energy transduction from molecule interactions into electrical signals, as well as their good biocompatibility. Usually the immobilization is performed under either potentiostatic or galvanostatic conditions in the presence of the enzyme in the monomer solution. Thus the enzyme is entrapped in the structure of the polymer and the film thickness can be easily controlled by using different quantity of electricity passed. In most cases, the polymer is assumed to function only as an inert matrix for the immobilization of enzymes. In the early applications, supporting electrolyte is often contained in the solution to increase the electroconductivity of solution and provides counter-ions for charge compensation. However, for ultra-thin PPy–GOD films, the additional supporting electrolyte was found to have lowered the potentiometric response of glucose [21]. This may be due to the reduction of the role of GOD as a counter-ion. Then, supporting electrolyte-free monomer solutions, as demonstrated by Adeloju et al. [22,23], can be considered to accomplish enzyme immobilization.

Generally the potentiometric glucose sensors can be based on the ion-selective electrodes (ISE) [13], the metal-oxide sensitive field effect transistor (MOSFET) [24], the ion-sensitive field effect transistor (ISFET) [25], the enzyme field effect transistor (ENFET) [26], and the light-addressable potentiometric sensor (LAPS) [27].

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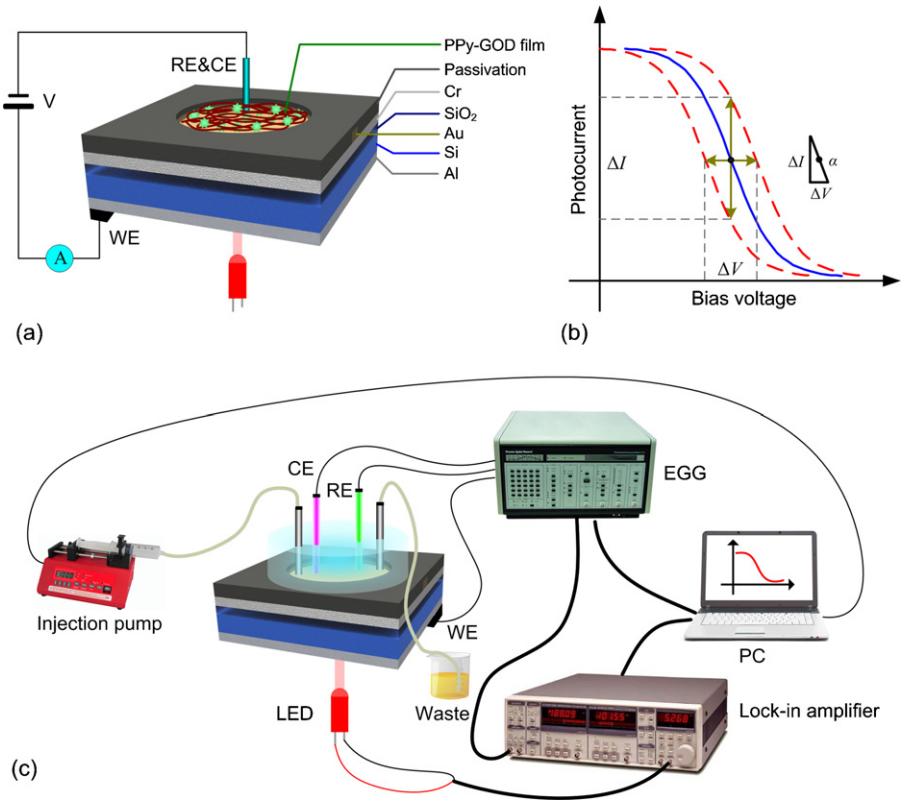

Fig. 1. Schematic diagram of experimental set up. (a) Sensor structure with the sequence of PPy–GOD/Au/Cr/SiO_2/Si; (b) characteristic IV curve of photovoltaic glucose sensor; (c) measurement system and flow injection system. WE: silicon substrate; RE: Ag/AgCl electrode; CE: Pt wire with a diameter of 0.5 mm.

Using the surface photovoltage technology employed in the light addressable potentiometric sensor [28], many biosensing devices are developed for enzyme based organic substances test [27,29,30]. As is known, gluconolactone ($C_6H_{10}O_6$) generated through the oxidation of glucose by GOD can be hydrolyzed and protons are produced. The basic principle of these biosensors lies in the pH variation induced by the enzymatic reactions as they are sensitive to pH change. However, the pH deviating from the optimum pH value would affect the enzyme activity to a certain extent, which in turn will impact the sample detection. In view of this, an electrolyte–metal–insulator–silicon structured sensor [31], which is sensitive to the redox potential determined by the ratio of oxidant and reductant in the analyte, has potential to overcome this problem.

In this study an EMIS sensor based on the surface photovoltage technology is constructed for rapid, long-time and continuous monitoring of glucose concentration. The potentiometric glucose sensor with a structure of GOD–metal–SiO_2–Si, is developed by modifying the metal layer of the sensor electrochemically with an ultra-thin PPy–GOD film in a supporting electrolyte-free monomer solution. The ultra-thin PPy–GOD film has been demonstrated to have good performance in glucose sensing [32]. With the application of the surface photovoltage technology, high sensitivity to surface potential change and continuous electrostatic measurement can be realized.

2. Experimental

2.1. Chemicals and reagents

All chemicals were of analytical grade. Glucose oxidase (Sigma G7141, 100,000–250,000 U/g) was from *Aspergillus niger*. The prepared GOD stock solution (500 U/mL) was stored at −20 °C. Pyrrole (from Aldrich) was distilled before use, stored in a refrigerator under a nitrogen atmosphere and protected from light. A β-D-glucose stock solution was stored and diluted to give the required concentrations. 0.05 M phosphate buffers (pH 7) were added to obtain standard glucose solutions. Phosphate buffers with different pH were prepared by neutralizing phosphoric acid with sodium hydroxide. Hydrogen peroxide solutions were also prepared with phosphate buffers. All solutions were prepared with deionized water.

2.2. Transducer preparation

The potentiometric transducer was fabricated with the layer sequence of Au/Cr/SiO_2/*n*-Si/Al. A sample *n*-type silicon wafer was etched in the middle region from the backside to obtain a 100-μm-thick silicon layer. Next, a silicon dioxide layer (50 nm) was formed on the polished frontside of the silicon through thermal oxidation. Then a Cr/Au layer (30/150 nm) was deposited on the silicon dioxide surface, followed by a photolithography and etching process to define the working electrode and lead wire. Afterwards an Al layer (300 nm) was evaporated onto the backside of the chip to form an ohmic contact. Finally epoxy resin was used as a passivation layer with the working electrode exposed.

2.3. Electropolymerization

In the electropolymerization process, the flat gold surface of the Au/Cr/SiO_2/*n*-Si/Al transducer was used as the working electrode. The exposed gold electrode had a diameter of 4 mm. Before immobilization of GOD, the sensor surface was thoroughly cleaned, and the prepared pyrrole monomer solution was purged with nitrogen for 20 min to remove traces of dissolved oxygen. Then the required quantity of GOD solution was added and mixed with

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