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On-chip highly sensitive saliva glucose sensing using multilayer films composed of single-walled carbon nanotubes, gold nanoparticles, and glucose oxidase

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ABSTRACT

It is very important for human health to rapidly and accurately detect glucose levels in biological environments, especially for diabetes mellitus. We proposed a simple, highly sensitive, accurate, convenient, low-cost, and disposable glucose biosensor on a single chip. A working (sensor) electrode, a counter electrode, and a reference electrode are integrated on a single chip through micro-fabrication. The working electrode is functionalized through a layer-by-layer (LBL) assembly of single-walled carbon nanotubes (SWNTs) and multilayer films composed of chitosan (CS), gold nanoparticles (GNp), and glucose oxidase (GOx) to obtain high sensitivity and accuracy. The glucose sensor has following features: (1) direct electron transfer between GOx and the electrode surface; (2) on-a-chip; (3) glucose detection down to 0.1 mg/dL (5.6 μM); (4) good sensing linearity over 0.017–0.81 mM; (5) high sensitivity (61.4 μA/mM-cm^2) with a small reactive area (8 mm^2); (6) fast response; (7) high reproducibility and repeatability; (8) reliable and accurate saliva glucose detection. Thus, this disposable biosensor will be an alternative for real time tracking of glucose levels from body fluids, e.g. saliva, in a noninvasive, pain-free, accurate, and continuous way. In addition to being used as a disposable glucose biosensor, it also provides a suitable platform for on-chip electrochemical sensing for other chemical agents and biomolecules.

1. Introduction

Being able to rapidly and accurately detect glucose levels in biological environments is of crucial importance to human health, especially in the condition of diabetes mellitus [1,2]. In the past several decades, various methods have been developed to measure glucose concentrations, including optical approaches (infrared (IR) spectroscopy, fluorescence spectroscopy, Raman spectroscopy, optical polarization rotation measurement, photo-acoustic probes, and surface plasmon resonance) [3,4], MEMS affinity sensing [5] and electrochemical methods [6,7]. Optical measurement approaches normally require very expensive instruments, significant processing time and highly trained professions; and affinity sensing method can run into nonspecific binding issues. It is the electrochemical glucose sensing that is most studied and demonstrated with high sensitivity, good accuracy, high selectivity, fast response time, low cost and many other outstanding properties [6,8].

Glucose sensing dates back to 1841 when glucose levels were measured through urine, but the correlation between urine and plasma glucose was later found inconsistent [9]. Until now, the monitoring of blood glucose levels has been the only recognized and widely used method for diagnosis and management of diabetes. However, users have to prick their fingers multiple times a day to use these devices, which are a major problem for young children and result in negative consequences for disease management. Finger pricking can also cause transient discomfort, bruise, fainting and blood-borne infection. A noninvasive and simple technique for diagnosis and monitoring of diabetes is thus very desirable. With a direct correlation between blood glucose and salivary glucose, it is possible to simply apply salivary glucose measurements to monitor individual's health conditions [10,11]. Hence, monitoring of salivary glucose levels can be an alternative prediagnostic method for diabetics and a health indicator for any individuals.

We have developed a simple, highly sensitive, accurate, convenient, low-cost, and disposable glucose biosensor on a single chip. It can effectively and reliably determine glucose concentrations in saliva. It is fabricated using micro-fabrication and LBL assembly procedures.

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The effective immobilization of enzymes onto the electrode surface has been one of the main factors that affect the sensing performance of an enzyme biosensor [12]. LBL assembly technique, among many enzyme immobilization methods, is proven to be a simple and effective method to prepare multilayer films containing the enzymes enabling good uniformity, stability, reproducibility and remarkable sensitivity [13,14].

Carbon nanotubes (CNTs) have been widely utilized as components for nanoscale electronic devices and biosensors due to their high electrocatalytic property, their ability to promote electron transfer, and their high thermal capacity. The CNTs-modified electrodes are reported to allow direct electron transfer (DET) to glucose oxidase [15,16]. The intrinsically hydrophobic carbon nanomaterials can decrease the bioactivity of GOx [17], however, functionalized carbon nanotubes have been proven to improve the glucose sensing performance and to facile direct electron transfer between GOx and the electrode surface [16,18].

Chitosan (CS), a linear polysaccharide, has a repeating hexosamide residue unit of one amino group and two hydroxyl groups permitting chemical modifications. It is nontoxic, biocompatible and economic. Chitosan is widely applied to immobilize biomolecules, especially in the assembly of enzymes and fabrication of amperometric biosensors, due to its excellent film forming and adhesion abilities, and its easiness for chemical modifications [19,20].

Gold nanoparticles (GNp) are very attractive in constructing electrochemical biosensors due to their unique physical and chemical properties. In particular, GNp are applied in electrode functionalization to catalyze electrode chemical reactions and conduct DET, and thus to increase the sensitivity of biochemical detection [21]. They are used as one negatively charged nanomaterial for electrostatic adsorption in LBL process. GNp not only increase the surface area to allow more enzyme to be immobilized, but also provide a mild microenvironment and give the biomolecules more freedom in orientation [22].

Tremendous work has been done to develop highly selective and sensitive glucose electrochemical biosensors, as reviewed by Professor Yao's group [23]. Glucose biosensors functionalized with CNTs or GNp have the ability to linearly detect glucose down to very low levels [24–29]. Chitosan, or polymers like poly(diallydimethylammonium chloride) (PDDA), poly(ethylenedioxithiophene) (PEDOT), has been commonly used in enzyme immobilization or as a linker molecule in the fabrication of amperometric biosensors [6,24,25,27–30]. However, we have developed an accurate and reliable saliva glucose sensor using direct electron transfer enabled by using SWNT.

Our on-chip electrochemical sensing device contains at least one working electrode, a counter electrode and a reference electrode. It is manufactured through several micro-fabrication procedures. The metal for all electrodes is Pt, which is widely applied for glucose sensing [20]. Pt provides significant advantages, such as much better conductivity, signal stability, and analytical response, over the other electrode materials, like Au, Ti, Ag [31,32]. Jin et al. have also proved that Pt's deposition onto the gold electrode can increase the electrocatalytic properties of the electrodes for glucose oxidation [33]. Pt can also be used for reference electrode [34]. Thus, it is highly feasible to integrate three Pt electrodes onto one single chip to realize on-chip electrochemical sensing. Further, this single chip can not only be used for glucose detection, but also provides an innovative platform for on-chip electrochemical sensing of other chemicals and biomolecules.

2. Experimental design and procedure

The chemicals and facilities used are listed here, and more importantly, we introduce the sensor fabrication and functionalization procedures. The sensor functionalization procedure can be modified to assemble other enzymes or antibodies in building a platform for on-chip electrochemical sensing of a wide range of chemicals and biomolecules.

2.1. Reagents and apparatus

Glucose oxidase (GOx, 17,300 units/G solid) from Aspergillus niger, gold nanoparticles (GNp, 20 nm diameter), chitosan (CS), poly(allylamine) (PAA, 20 wt% solution in water), acetate buffer solution (pH 4.65), D-(+)-Glucose, phosphate buffered saline (PBS, pH 7.4) were purchased from Sigma Aldrich. Carboxyl (COOH) groups functionalized single-walled carbon nanotube suspension (SWNT, diameter: 1–2 nm; length: 2–5 μm, 4000 mg/L in distilled (DI) water with ~5–7 wt% COOH groups at the end) was purchased from Brewer Science Company. Dulbecco's phosphate-buffered saline (DPBS, no calcium, no magnesium) was purchased from Life Technologies.

Silicon wafers (diameter 3″, boron doping, ⟨100⟩ orientation, resistivity 0–100 Ω, thickness 406–480 μm, one-side polished) were purchased from University Wafer; and Platinum Pellets (1/8″ Diameter × 1/8″ Length, Per Gram, 99.99% pure) were purchased from Kurt J. Lesker Company.

Facilities used in Gorge J. Kostas Nanoscale Technology and Manufacturing Research Center include wet bench wafer cleaning system, Bruce furnace 7355B (oxidation), Nanospec thickness measurement machine, Brewer/laurell spinner, Quintel 4000 mask aligner, Unaxis ICP etch (Plasma Therm 790), Electron-beam deposition system, Micro automation 1006 dicing saw, and Supra 25 SEM.

All cyclic voltammetry (CV) and amperometric measurements were performed in PBS (0.1 M, pH7.4) at room temperature (~23 °C) using the DY2113 mini potentiostat from Digi-Ivy company. The adapter between sensor and the potentiostat was purchased from DropSens Company. A Supra 25 Scanning Electron Microscope (SEM) was employed for the surface morphological characterization of SWNT, GNp and GOx on the sensor electrode reactive area.

2.2. Device fabrication

Fabrication of the disposable glucose biosensor is described in this section. It includes micro-fabrication of the sensor chip and LBL assembly for electrode modification.

The on-chip electrochemical sensing device contains at least one working electrode, a counter electrode and a reference electrode (one possible electrode configuration – Fig. 1a). The small rectangle (purple) marks out the reactive area on the working electrode while the larger one (blue) indicates where sample drops on. One such device- S2D2 is of size $20 \times 10\ mm^2$ with the reactive area $32\ mm^2$. It can be manufactured through microfabrication (Fig. 1b). Starting from a pre-cleaned silicon wafer (~500 μm thick), the surface is oxidized in wet atmosphere (Bruce Furnace 7355B) at 1100 °C for 40 min to form a 0.5 μm thick SiO_2 layer as the insulator layer. Then, photolithography is conducted to create the pattern of desired microelectrodes using MICROPOSIT S1813 photoresist. 200 nm thick Pt (Platinum) with 20 nm thick Cr (Chromium) adhesive layer is deposited on the surface through E-beam evaporation. After lifting off the extra Pt by acetone, isopropyl alcohol (IPA) and DI water and dicing the wafer into small chips ($20 \times 10\ mm^2$), the electrode system is present on each chip.

The glucose biosensor is fabricated through a LBL assembly of SWNT and multilayer films composed of CS–GNp–GOx (Fig. 1c) [35]. The CS–GNp–GOx unit can be repeated several times to