Electrochimica Acta 174 (2015) 574–580

Contents lists available at ScienceDirect

Electrochimica Acta

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

A Miniature Graphene-based Biosensor for Intracellular Glucose Measurements

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ARTICLE INFO

Article history:
Received 12 March 2015
Received in revised form 20 May 2015
Accepted 8 June 2015
Available online 10 June 2015

Keywords:
Graphene
Bio-sensors
Glucose-oxidase
Intracellular sensors
Graphene oxide
Glucose Sensor

ABSTRACT

We report on a small and simple graphene-based potentiometric sensor for the measurement of intracellular glucose concentration. A fine borosilicate glass capillary coated with graphene and subsequently immobilized with glucose oxidase (GOD) enzyme is inserted into the intracellular environment of a single human cell. The functional groups on the edge plane of graphene assist the attachment with the free amine terminals of GOD enzyme, resulting in a better immobilization. The sensor exhibits a glucose-dependent electrochemical potential against an Ag/AgCl reference microelectrode which is linear across the whole concentration range of interest (10 – 1000 μM). Glucose concentration in human fat cell measured by our graphene-based sensor is in good agreement with nuclear magnetic resonance (NMR) spectroscopy.

1. Introduction

Graphene has been enticing much attention due to its fascinating properties, such as extremely large surface area ($2630\,m^2\,g^{-1}$), high optical transmittance (~ 97.7 %), large mechanical fracture strength, (~ 125 GPa), excellent thermal conductivity (~ $5000\,W\,m^{-1}\,K^{-1}$) and superb charge-carrier mobility (~ $200{,}000\,cm^2\,V^{-1}s^{-1}$) [1–8]. Its remarkable properties have opened up a whole realm of applications [3,8–11], amongst which biosensing has been capturing a huge interest [12–14]. This is mainly because of the great promise and performance owning to its tremendously large surface area to volume ratio as a dominating and favorable parameter for sensing applications [8,15,16]. The sensing is usually achieved via immobilization of enzymes onto the sensing surfaces of a suitably modified electrode, which can provide highly selective, sensitive, and rapid analysis of various biological species like DNA [17], Human IgG [18] and glucose [19]. Carbon nanotube (CNT) modified electrodes have already been very successful for biosensing of glucose, which is realized by a dramatic decrease in the hydrogen peroxide overpotential, plus the direct electron transfer of glucose oxidase (GOD) observed at as-made electrodes [20,21]. However, using graphene for such applications can offer several advantages over CNTs for its superior properties: Its 2-dimentional nature (single atomic layer of graphite) can maximize the interaction between the surface dopants and adsorbates. It offers much lower Johnson noise and higher sensitivity [22–24], as compared to CNT, therefore, a minute variation of carrier concentration can cause a notable variation of electrical conductivity [25]. Moreover, graphene can be obtained via chemical conversion of graphite, which is a simple and inexpensive synthesis method [26].

We employ the GOD enzyme, which belongs to a family of oxidases that are widely used in many glucose sensors. The binding between GOD and the electrode surface is a critical parameter for the stability and sensitivity of the biosensor. Therefore, it is important to select a suitable electrode material that can favorably bind an enzyme. Various methods, such as covalent binding [27], embedding [28], and cross-linking [29–31], have already been exploited to immobilize the GOD onto different supporting materials. Functionalized graphene has reactive functional groups that can covalently attach with the free NH_2-terminals of the enzyme/protein to give an amide linkage [32]. Nevertheless,

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http://dx.doi.org/10.1016/j.electacta.2015.06.035
0013-4686/

covalent bonding is not obligatory for irreversible protein adsorption to surfaces. There are instances where proteins are partially denatured as they rearrange upon approaching a hydrophobic surface, yet, retaining their enzymatic activity. In general, the enzymatic activity is realized through immobilization of GOD either onto a hydrophilic microenvironment by the covalent attachment method or onto a hydrophobic microenvironment by physisorption. It has been found that the latter microenvironment offers a higher specific activity as compared to the former [33]. Since, graphene is extremely hydrophobic [34], we anticipate good immobilization through physisorption onto its surface that should result in better sensing properties.

Recently, a graphene based biosensor has been reported by utilizing polypyrrole (Ppy) to capture the graphene-oxide (GO) and GOD onto the glassy carbon electrode surface [19]. We report a biosensor merely based on graphene, using a graphene coated pipette as a substrate that was inserted into the intracellular environment of a single human fat cell. The diameter of working and the reference electrode was ~0.7 mm. To our knowledge this is the first time graphene has been used for this purpose.

In this paper, we describe a method in which the GOD is immobilized onto the graphene coated pipette surface (working electrode), shown in Fig. 1. The working electrode was prepared by coating it with commercial graphene-based conductive ink, which can suitably capture GOD on its surface. The peptide bonding between functionalized graphene and GOD molecules provides a biocompatible microenvironment to retain the enzyme in its native structure [35]. Moreover, functionalized graphene nanosheets within the conjugate facilitate the electron transfer between the matrix and electroactive center of the GOD, and the percolating network of graphene nanosheets provides multiplexed paths for the rapid charge conduction [36,37]. Furthermore, the amount of GOD that binds to the graphene offers a substantial loading, well enough for these enzymatic biosensing applications [38]. Subsequently, the entire electrode assembly is employed for the detection of intracellular glucose, which is in good agreement with other techniques such as nuclear magnetic resonance spectroscopy (NMR).

2. Experimental

2.1. Electrochemical Setup

All experiments were carried out with a conventional two-electrode system. The working electrode was a graphene coated glass pipette with diameter of 0.7 μm, modified with graphene-GOD composite. The potential of the working electrode was measured against the reference electrode of Ag/AgCl saturated with 3.0 M KCl. All electrochemical experiments were performed at room temperature.

2.2. Synthesis of Graphene

Graphite-oxide was made by the modified Hummer's method [10,39], and mixed with water to yield a yellow-brown suspension. This suspension was then ultrasonicated and treated with hydrazine hydrate. The mixture was heated in an oil bath at about 100 °C in a water-cooled condenser for about 24 h. This resulted in the formation of GO which was precipitated as a black solid. This black solid was subsequently filtered and washed with deionized

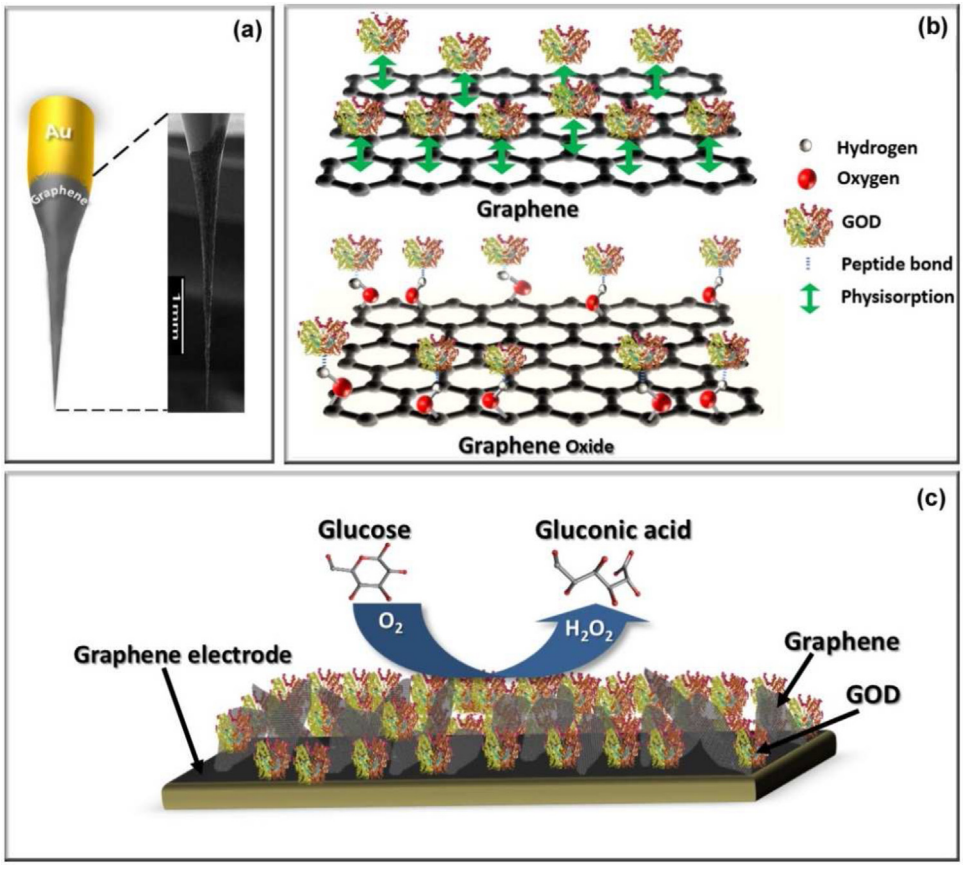

Fig. 1. a) Schematic and SEM image of the pipet tip used as platform for enzyme immobilization and potentiometric detection of intracellular glucose, **b**) Schematic illustration of the GOD immobilization on graphene with peptide bond formation between carboxylic groups of GO and GOD and prospective physisorption of GOD on hydrophobic surface of graphene, and **c)** Representation of the GOD entrapment within a graphene matrix, along with possible electrochemical reaction near the electrode.