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Glucose sensor based on redox-cycling between selectively modified and unmodified combs of carbon interdigitated array nanoelectrodes

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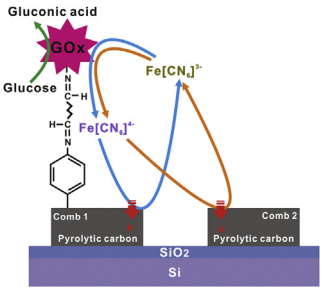
HIGHLIGHTS

- Fabrication of a novel IDA-nanoelectrode-based glucose sensor by using the cost-effective, easy and simple C-MEMS technology.
- Reproducible and selective enzyme immobilization at a designated comb of IDA nanoelectrodes.
- Electrochemical-enzymatic redox cycling between glucose oxidase and the two comb-shaped nano-electrodes of the IDA.
- Reducing the electrode-to-electrode gap between the two combs increases the diffusion flux of redox species.
- Enhancement of sensitivity (~2.3 times) and LOD (~295 times) at the unmodified comb compared to the modified comb.

GRAPHICAL ABSTRACT



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ABSTRACT

We present a novel electrochemical glucose sensor employing an interdigitated array (IDA) of 1:1 aspect ratio carbon nanoelectrodes for the electrochemical-enzymatic redox cycling of redox species (ferricyanide/ferrocyanide) between glucose oxidase (GOx) and the two comb-shaped nanoelectrodes of the IDA. The carbon nanoelectrodes were fabricated using a simple, cost-effective, reproducible microfabrication technology known as the carbon-microelectromechanical-systems (C-MEMS) process. One comb (comb 1) of the IDA was selectively modified with GOx via the electrochemical reduction of an aryl diazonium salt, while the other comb (comb 2) remained unmodified; this facilitates electrochemically more active surface of comb 2, resulting in sensitive glucose detection. Ferricyanide is reduced to ferrocyanide by the GOx in the presence of glucose, and ferrocyanide diffuses to both combs of the IDA where it is oxidized. The limited electrochemical current collection at the surface-modified comb 1 is counterbalanced by the efficient redox cycling between the enzyme sites at comb 1 and the bare carbon surface of comb 2. Reducing the electrode-to-electrode gap between the two combs (gap = 1.9 μm) increases the diffusion flux of redox species at comb 2 hence, enhanced the sensitivity and limit of

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detection of the glucose sensor by ~2.3 and ~295 times, respectively at comb 2 compared to comb 1. The developed IDA-based glucose sensor demonstrated good amperometric response to glucose, affording two linear ranges from 0.001 to 1 mM and from 1 to 10 mM, with limits of detection of 0.4 and 61 μM and sensitivities of 823.2 and 70.0 μA mM^{-1} cm^{-2}, respectively.

1. Introduction

Interdigitated arrays (IDA) of microelectrodes have been of particular interest because of their advantages in electrochemical sensing, such as high current densities, low charging currents, and reduced solution resistance effects [1]. In addition, when microelectrodes are scaled down to the nanoscale, mass transport and steady-state rates are enhanced, resulting in fast response times. IDA nanoelectrodes have intrinsically enhanced sensitivity based on the recycling of the redox species between their differently biased comb-shaped electrode pairs. The electrode pairs are tightly spaced so that redox species experience multiple redox reactions on the electrode surfaces before diffusion into the bulk solution; this result in electrochemical current amplification compared to the case in which both electrode pairs are biased at the same potential. The electrochemical current amplification or redox cycling of the IDA nanoelectrodes is largely dependent on the geometric features of the electrodes, including the electrode spacing, width, and aspect ratio. Reducing the width and gap between the IDA nanoelectrodes to sub-micrometer enhances the diffusion flux of the redox species, leading to enhanced redox cycling, decreased equilibration time, and increased collection efficiency [2]. In addition, an increase in the electrode aspect ratio can improve the current amplification of IDA nanoelectrodes by enhancing diffusion between the electrode sidewalls and increasing the overall area of the electrode surface [3]. Interdigitated microelectrodes with a high aspect ratio fabricated using selective deposition of a conductive layer onto a pre-patterned silicon structure also showed high signal amplification, comparable to that of nano-gap IDA devices [4]. Recently, our group demonstrated the simple fabrication of carbon IDA nanoelectrodes with 1:1 aspect ratios using carbon-microelectromechanical system (C-MEMS) technology; this nanoelectrode architecture successfully achieved current amplification as high as 1,116 times in a microchannel [5].

The selective [6] and sensitive [7] electrochemical detection of redox species has been demonstrated using IDA microelectrodes. Furthermore, arrays of sub-micrometer-spaced IDAs have been used for the determination of alkaline phosphatase and β-galactosidase enzyme activities [8]. IDA microelectrodes have been applied in electrochemical enzyme immunoassays for the detection of 4-aminophenol through its redox cycling between IDA nanoelectrodes [9]. In addition, electrochemical sensors based on IDAs for the detection of glucose, urea, and triglycerides have also been developed [10,11]. Recently, IDA microelectrodes were reported as an enzyme-based glucose biosensor platform with good sensitivity and selectivity [12].

For sensor systems based on IDA electrodes, enzymes are commonly immobilized by approaches that employ conducting polymers [10], sol–gel methods [12], and self-assembled monolayers (SAMs) [13]. Three-dimensional IDAs were reportedly modified with glucose oxidase (GOx) using the layer-by-layer self-assembly of cationic polyethyleneimine [14]. Jin et al. reported the selective immobilization of GOx on gold IDA microelectrodes through the adsorption and desorption of SAMs of alkanethiols. However, SAMs can exhibit disadvantages such as poor long-term stability, limited potential windows [15], and desorption at the reductive or oxidative potential [16]. Therefore, the selective, stable modification of electrode surfaces is a critical issue for the development of biological sensors based on IDA electrodes. To address this issue, aryl diazonium salts were identified as possible alternatives to SAMs due to the highly stable, selective, and controlled grafting of functional groups on the electrode surface [17–19]. The electrochemical reductions of 4-carboxyphenyldiazonium [20], 4-nitrophenyl diazonium [21], Nile blue diazonium [22], and 4-aminophenyldiazonium [23] salts have been used for the immobilization of biomolecules. Harper et al. reported the selective functionalization of pyrroloquinoline quinone on a closely spaced gold disk electrode array using the 4-nitrophenyl diazonium salt for the detection of nicotinamide adenine dinucleotide (NADH) oxidation [24]. In addition, DNA sequences and proteins were covalently immobilized on individually addressable gold disk electrodes by the electro-deposition of carboxyphenyl diazonium salts [25].

To date, most of the IDA microelectrodes used for the detection of biomolecules have been based on gold [13] and platinum [8]. These noble metal electrodes generally require a metal adhesion layer such as Ti, Cr, or Ta. However, these dual metal layers are degraded via galvanic corrosion when placed in contact with the electrolyte. In order to overcome these issues, in the present work, adhesion-layer-free pyrolytic carbon IDA nanoelectrodes were fabricated using a simple batch microfabrication technology known as the C-MEMS process, which is highly reproducible, easy, and cost-effective. Unlike other microfabrication methods this process consists only of conventional photolithography followed by pyrolysis that converts the photoresist patterns into 3-D glassy carbon nanoelectrodes. The carbon obtained via this method features excellent physicochemical properties, outstanding chemical, electrochemical stability and high biocompatibility. In addition, using the C-MEMS process the aspect ratios of the carbon nanoelectrodes can also be controlled and that attributes to high redox-cycling efficiency [3]. By selectively modifying one comb of the carbon IDA nanoelectrodes via the electrochemical deposition of the 4-nitrophenyl moiety and immobilization with GOx, we could prepare two types of combs in one IDA electrode set: one comb covalently immobilized with GOx (comb 1) and the other comb without GOx (comb 2) as illustrated in Fig. 1A. The high redox-cycling efficiency and selective immobilization in proximity of working electrodes enabled highly sensitive glucose sensing. The electrochemical detection of glucose was performed using ferricyanide ($[Fe(CN)_6]^{3-}$) as a redox mediator to prevent electrode fouling and enhance redox cycling. Many glucose biosensors use natural O_2 as the electron acceptor for the generation and detection of H_2O_2 [26]. The O_2-mediated glucose sensors suffer from interference because of the similar oxidation potentials of ascorbic acid and uric acid to that of H_2O_2.

This study proposes a new IDA-based glucose sensor using the electrochemical-enzymatic redox cycling of $[Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$ between the GOx and both the combs of the 1:1 aspect ratio carbon IDA nanoelectrodes. The modification strategy of IDA nanoelectrodes (comb 1) was made simple by using an easy and