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Highly sensitive non-enzymatic electrochemical glucose biosensor using a photolithography fabricated micro/nano hybrid structured electrode

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ABSTRACT

In this study, a novel non-enzymatic glucose biosensor based on a simple photolithographic process is proposed. To fabricate the sensor, photoresist AZ-1518 was spin-coated onto a reclaimed silicon wafer, and then, a mask with a hexagonal close-packed circle array was employed for exposure and development to generate a hexagonal close-packed column array of AZ-1518. The diameter of each circle was set as 3 μm. Subsequently, a thermal melting process was employed to convert each photoresist column into a photoresist hemisphere. A gold thin film was then sputtered onto the hemisphere array of AZ-1518 to form the sensing electrode. Finally, gold nanoparticles were deposited onto the gold thin film using a self-assembly monolayer method to enhance the sensing area. Measurements showed a 10.2-fold enhancement of the sensing area in comparison with a plain gold electrode. Actual detection of glucose demonstrated that the proposed non-enzymatic glucose biosensor can operate in a linear range of 55.6 μM–13.89 mM. It had a sensitivity of 749.2 $\mu A\,mM^{-1}\,cm^{-2}$ and a detection limit of 9 μM. The novel glucose biosensor proposed here has several advantages such as being enzyme free, simple to fabricate, low cost, and easy to preserve on a long-term basis. Thus, it can feasibly be used for future clinical applications.

1. Introduction

Diabetes is now a severe global public health problem. It can be classified into two main types: type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). T1DM is caused by the insufficient production of insulin in the pancreas, whereas T2DM occurs when the body does not make full use of the insulin produced by the pancreas [1]. As of 2015, the International Diabetes Federation reported that approximately 387 million people worldwide suffer from diabetes, with 90% of the cases suffering from T2DM [2,3]. Regular detection of a diabetes patient's blood glucose is essential for effectively maintaining their blood sugar level.

Various techniques have been proposed for continuous glucose monitoring. In general, electrochemical and optical approaches are the most commonly used methodologies [4]. Electrochemical sensors make up the majority of commercially available glucose-sensing devices because of their practicability, simplicity, and low cost [5,6]. Electrochemical-based sensors can be classified according to the use of enzymatic and non-enzymatic approaches. In enzymatic approaches, glucose is oxidized to gluconolactone, with the reaction catalyzed by the glucose-specific enzyme glucose oxidase (GOx). In non-enzymatic approaches, glucose is directly oxidized to gluconolactone at nanostructured electrodes that provide a large reaction area for effective electrocatalytic activity. The advantages of enzymatic approaches include their high response to glucose and good specificity of glucose detection [7–9]; however, the inevitable disadvantages include the relatively complicated and multistep immobilization processes, chemical and thermal instability, and degradation of the GOx. Therefore, non-enzymatic approaches, which do not require an immobilization procedure, are free from the degradation problem, and have high stability,

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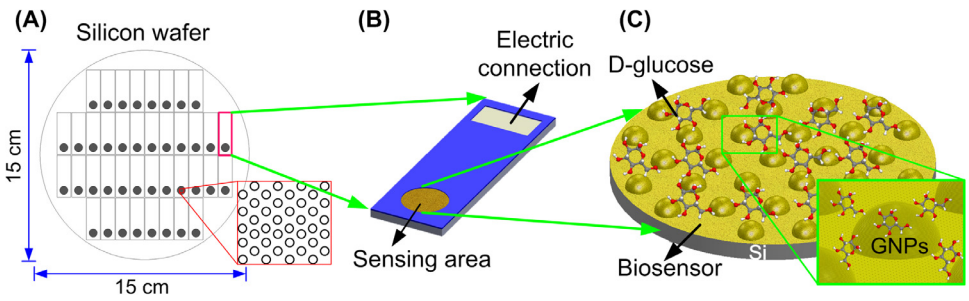

Fig. 1. Schematic of the proposed novel non-enzymatic biosensor. (A) Mask layout, (B) sensor packing, and (C) sensing electrode with a micro/nano hybrid structure.

simplicity, and reproducibility, have recently attracted increasing attention [10,11].

Gold is generally recognized as an inert metal since electrons fill the *d* orbitals. Therefore, in electro-oxidation, gold is usually considered as a poor substrate to absorb the participating species of organic substances, such as glucose. However, nanoscale gold materials, which have catalytic mechanisms similar to natural GOx, can provide a strong catalytic activity [12]. Because gold is an inert metal, a glucose biosensor based on a gold substrate is more stable given variations in pH and temperature. Feng et al. developed a non-enzymatic glucose sensor using a glassy carbon electrode (GCE) coated with a chitosan-gold nanoparticles (GNPs) nanocomposite-sensing film [13]. This sensor produced a linear performance in the concentration range from 400 μM to 10.7 mM and had a 370-μM limit of detection. Li et al. employed specific boronic acid-diol binding to develop a non-enzymatic platform for glucose sensing [14]. They used a GNPs-Prussian blue (GNPs-PB) nanocomposite deposited on a gold electrode surface as the electrochemical indicator. A second GNP layer was deposited on the GNPs-PB nanocomposite surface to further improve the sensitivity of the sensor. The sensor had a linear detection range of 0.1–13.5 μM and a 0.05-μM detection limit.

Besides the use of GNPs for glucose detection, other gold nanostructures have also been applied to glucose biosensors. In one study, urchin-like gold submicrostructures, which have better catalytic activity for glucose oxidation than flower-like gold submicrostructures, and Nafion solution were cast onto a GCE for non-enzymatic glucose detection [15]. The linear detection range, sensitivity, and detection limit were measured as 0.2–13.2 mM, 16.8 $\mu A\,mM^{-1}\,cm^{-2}$, and 10 μM, respectively. Chen et al. reported a non-enzymatic electrochemical glucose biosensor based on nanoporous gold (NPG) [16]. Since silver can be etched off by a HNO_3 solution, such a solution was employed to etch off the $Au_{35}Ag_{65}$ (at.%) alloy of Ag to form a NPG film and then to directly sense glucose concentration. The linear range, sensitivity, and detection limit of this sensor were measured as 1–18 mM, 20.1 $\mu A\,mM^{-1}\,cm^{-2}$, and 800 nM, respectively. Shu et al. proposed a dendrite-like electrode of gold nanostructures (DGNs) to directly detect glucose levels [17]. The electrode was immersed in a $HAuCl_4$ solution and a potential was applied to form DGNs on a GCE. This electrode had a wide linear detection range of 0.1–25 mM, a high sensitivity of 190.7 $\mu A\,mM^{-1}\,cm^{-2}$, and a low detection limit of 0.05 μM. Cherevko and Chung investigated a gold nanowire array electrode for use as a non-enzymatic glucose biosensor [18]. Specifically, a gold nanowire array was deposited on an anodic aluminum oxide (AAO) template, and then the AAO template was removed using a NaOH solution to obtain a nanowire structure array. The linear detection range of this device was 0.5–14 mM, while the sensitivity and detection limit were 309 $\mu A\,mM^{-1}\,cm^{-2}$ and 30 μM, respectively. In addition to the pure gold-based sensors, composite metals have recently been studied and used in non-enzymatic glucose biosensors. For example, Shen et al. proposed a bimetallic Pd–Au cluster non-enzymatic glucose biosensor that was synthesized through a direct chemical reduction method [19]. This sensor demonstrated a linear range of 0.1–13.5 μM, a sensitivity of 75.3 $\mu A\,mM^{-1}\,cm^{-2}$, and a detection limit of 50 μM.

The trend for development of in vitro diagnosis devices requires that these devices be low cost, sensitive, specific, easy to use, and disposable. For the current non-enzymatic glucose detection techniques, a relatively complicated and time-consuming nanomaterial

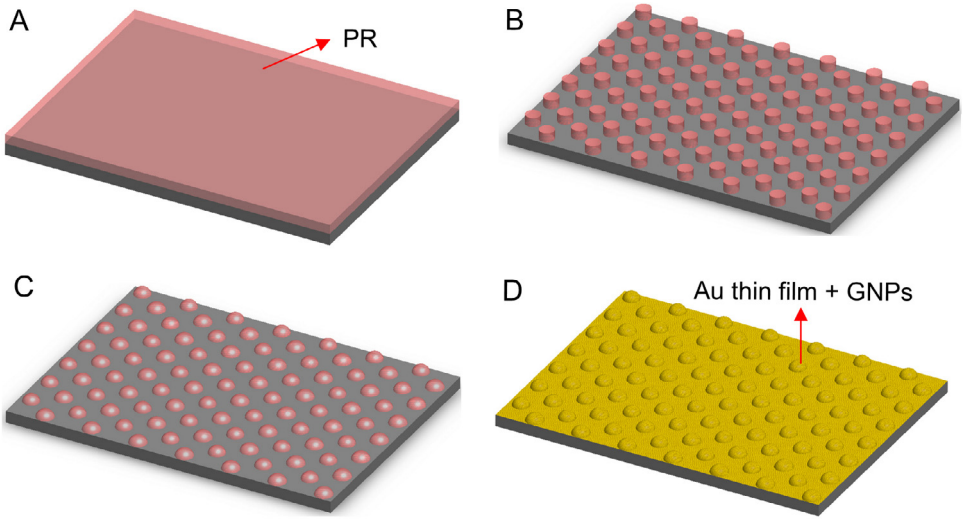

Fig. 2. Schematic of the fabrication procedure. (A) Silicon-wafer cleaning and positive photoresist coating, (B) exposure and development, (C) thermal melting, and (D) gold thin film sputtering and deposition of GNPs.