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A novel paper-based device coupled with a silver nanoparticle-modified boron-doped diamond electrode for cholesterol detection

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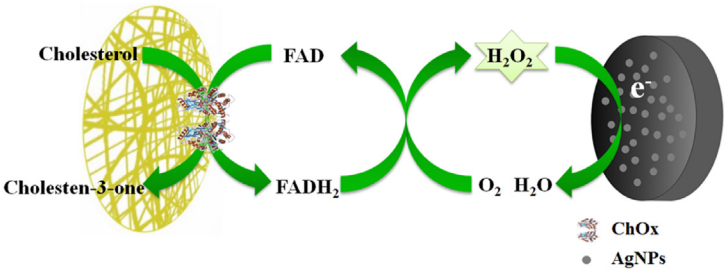
HIGHLIGHTS

- Novel PAD coupled with AgNP/BDDE for cholesterol determination was developed.
- Wide linear range, low detection limit and high selectivity were achieved.
- This sensor was successfully applied for cholesterol determination in bovine serum.
- This platform offers the advantages of low sample/reagent consumption and low cost.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel paper-based analytical device (PAD) coupled with a silver nanoparticle-modified boron-doped diamond (AgNP/BDD) electrode was first developed as a cholesterol sensor. The AgNP/BDD electrode was used as working electrode after modification by AgNPs using an electrodeposition method. Wax printing was used to define the hydrophilic and hydrophobic areas on filter paper, and then counter and reference electrodes were fabricated on the hydrophilic area by screen-printing in house. For the amperometric detection, cholesterol and cholesterol oxidase (ChOx) were directly drop-cast onto the hydrophilic area, and H_2O_2 produced from the enzymatic reaction was monitored. The fabricated device demonstrated a good linearity (0.39 mg dL^{-1} to 270.69 mg dL^{-1}), low detection limit (0.25 mg dL^{-1}), and high sensitivity (49.61 μA mM^{-1} cm^{-2}). The precision value for ten replicates was 3.76% RSD for 1 mM H_2O_2. In addition, this biosensor exhibited very high selectivity for cholesterol detection and excellent recoveries for bovine serum analysis (in the range of 99.6–100.8%). The results showed that this new sensing platform will be an alternative tool for cholesterol detection in routine diagnosis and offers the advantages of low sample/reagent consumption, low cost, portability, and short analysis time.

1. Introduction

Cholesterol is an essential component of the mammalian cell membrane and is a precursor of bile acid and steroid hormones. However, abnormal cholesterol levels are related to several diseases, such as hypertension, coronary heart disease, arteriosclerosis, brain thrombosis, lipid metabolism dysfunction and myocardial infarction

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[1]. Thus, the monitoring of the cholesterol level is very important for disease control and prevention. Various analytical strategies for cholesterol assays have been developed that can be sub-classified into two groups, including a chemical and an enzymatic method [2–5]. Currently, the enzymatic method has received greater attention for cholesterol analysis because it provides greater sensitivity and selectivity than the chemical method. The well-known principle of an enzyme-based cholesterol sensor is typically based on the reaction between cholesterol and the cholesterol oxidase (ChOx) enzyme. Free cholesterol is oxidized by cholesterol oxidase to produce 4-cholestene-3-one and hydrogen peroxide (H_2O_2), and the H_2O_2 generated can be used for indirect quantification of cholesterol [6–8]. The electrochemical method, especially the electrochemical biosensor, is extremely attractive for the determination of cholesterol because the electrochemical biosensors provide high sensitivity, require inexpensive instrumentation, and are suitable for real-time analysis. Previously, several methods have been used to immobilize the enzyme onto an electrode, such as entrapment in a polymer [9], covalent linkage with glutaraldehyde [10] or a polymer [11], and electrostatic adsorption [12]. Although these sensing platforms provide good performance for cholesterol detection, limitations still remain in terms of time consumption, detection performance and the ease with which the enzyme is denatured during its immobilization. To overcome this drawback, it is necessary to design and fabricate a new cholesterol sensor that offers simple fabrication, an easy immobilization step, and a short analysis time.

Recently, filter paper has received interest as a potential material for sensor applications due to its large surface area and low cost. In 2009, electrochemical detection on a paper-based analytical device (PAD) was first developed by Dungchai and coworkers [13]. This platform became a new alternative analytical device and was applied in many application areas [14–19]. Their simplicity of fabrication, portability and disposability coupled with the small volume of reagent/sample solution required for PADs make them suitable and very appealing for the development of new clinical diagnostic tools. Moreover, the biocompatibility of filter paper makes it suitable for immobilization of enzymes or other bioreceptors [20–22]. However, the traditional paper-based electrochemical devices with a three-electrode system (consisting of a working electrode (WE), a counter electrode (CE), and a reference electrode (RE)) still have some limitations, including low sensitivity because of a small electrode area and the use of carbon ink as a working electrode [13,23].

To solve these problems, the use of a commercial electrode coupled with PAD was considered. Boron-doped diamond (BDD) thin films are novel carbon-based materials that have very attractive properties compared to other conventional electrodes in terms of (i) wide electrochemical potential window; (ii) low and stable background current; (iii) resistance to electrode fouling; (iv) high response reproducibility and long-term response stability; and (v) biocompatibility. As advantages, we can conclude that the BDD electrodes provide the potential for electroanalysis application with high sensitivity [24–26]. To eliminate possible interference from easily oxidizable species in biological samples, cholesterol detection and measurement via the monitoring of H_2O_2 reduction is proposed in this work. Unfortunately, the reduction of H_2O_2 cannot be observed on a bare BDD electrode. Therefore, silver nanoparticles (AgNP) have been considered as the modifier for modification of the BDD surface because they have a large specific surface area, excellent conductivity and extraordinary electrocatalytic activity. Moreover, AgNP also show an excellent electrocatalytic activity for H_2O_2 reduction [27–30].

On this basis, we have developed a novel cholesterol biosensor based on a silver nanoparticle-modified boron-doped diamond (AgNP/BDD) electrode with PAD. An electrodeposition method was used to deposit AgNP onto the BDD electrode surface. The resulting device possessed the advantages of simple fabrication, small sample volume/reagent requirements, low cost and short analysis time. In addition, the AgNP/BDD electrode exhibited a highly sensitive and selective response to detect cholesterol without disturbance from interfering compounds such as ascorbic acid, uric acid, and glucose. To the best of our knowledge, this the first report of the coupling of an AgNP/BDD electrode with PAD to fabricate a cholesterol biosensor. The results show that this device displayed excellent performance, a wide linear range, and a low detection limit for cholesterol detection.

2. Experimental

2.1. Chemicals and materials

Cholesterol and cholesterol oxidase (ChOx) from *Streptomyces* sp. (25 U/mg) and lipid cholesterol enriched from adult bovine serum were purchased from Sigma (St. Louis, MO). Potassium dihydrogen phosphate (KH_2PO_4) was purchased from Carlo Erba Reagenti-SpA (Val de Reuil, France). Hydrogen peroxide (H_2O_2), disodium hydrogen phosphate (Na_2HPO_4), potassium chloride (KCl) and boric acid (H_3BO_3) were purchased from Merck (Darmstadt, Germany). Silver nitrate ($AgNO_3$) was purchased from POCH S.A. (Poland). Glacial acetic acid (CH_3COOH) was purchased from Fisher Scientific (Pittsburgh, PA). Phosphoric acid (H_3PO_4, 85%) was purchased from Carlo Erba (Rodano, MI, USA). Carbon ink and silver/silver chloride (Ag/AgCl) ink were obtained from Gwent Group (Torfaen, United Kingdom). Filter paper grade no. 1 (size, 46 × 57 cm^2) was purchased from Whatman. A stock solution of cholesterol was prepared daily by dissolving cholesterol in a mixture of Triton X-100 and isopropanol. This stock solution was further diluted to make different concentrations of cholesterol in 0.05 M PBS pH 7.4. The solution was stirred with a magnetic bar at 60 °C to obtain a homogeneous solution. Stock solution of ChOx was freshly prepared by dissolving in 0.05 M phosphate buffer (pH 7.0). All chemicals were used without further purification.

2.2. Sample preparation

The labeled concentration of cholesterol in adult bovine serum was used to represent the serum sample. The serum samples were prepared by dissolving adult bovine serum powder in Triton X-100 and isopropanol, and diluting with 0.05 M PBS pH 7.4. The solution was stirred with a magnetic bar at 60 °C.

2.3. Preparation of AgNP/BDD electrode

A simple one-step electrodeposition method was used for the electrode modification. A three-electrode system (Ag/AgCl (3 M KCl) electrode as the reference electrode (RE), a platinum wire as the counter electrode (CE) and a BDD electrode as the working electrode (WE)) was used in this work. Electrodeposition of AgNPs onto the BDD electrode surface was accomplished by dipping the electrode in a solution of $AgNO_3$ in Britton–Robinson buffer (pH 2) and applying a potential with stirring. The electrode was then rinsed with water to remove any physically adsorbed substances on the electrode.

The electrodeposition parameters for the modification of the electrode including deposition potentials, deposition times, and

Table 1
Summary of the experimental parameters for modification of electrode.

Parameters	Range tested	Optimum value
deposition potentials (V vs. Ag/AgCl)	(−0.3) to (−0.7)	−0.5
deposition times (s)	25–300	150
concentrations of $AgNO_3$ (mM)	0.25–10	5