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Research paper

Development of novel paper based electrochemical immunosensor with self-made gold nanoparticle ink and quinone derivate for highly sensitive carcinoembryonic antigen

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ABSTRACT

In this study, a low cost and robust paper based electrochemical immunosensor was developed using self-made gold nanoparticle electrode for rapid and selective detection of Carcinoembryonic antigen (CEA). The working and counter electrode were printed using gold nanoparticle ink while the reference electrode was made using commerical silver ink. A simple mercapto-amine functionalised receptor (**R1**) based on moiety Quinone compound was formed on the paper based screen-printed gold (Au) electrode (P-SPGE) for highly selective sensing of CEA. A self-assembled monolayer principle (SAM) was exploited to fabricate the sensitive and selective immunosensor electrodes. In electrode fabrication, SAM layer was accomplished using covalent linkage of thiol in the **R1** compound on the P-SPGE surface. Immobilised amine served as a layer for further binding of biological components. CEA levels were examined quantitatively using differential pulse voltammetry (DPV) and limit of detection (LoD) was calculated as 0.33 ng mL^{-1}. The performance of P-SPGE was compared with the commercial screen-printed Au electrode. A novel P-SPGE functionalised with **R1** exhibited the tremendous performance for CEA detection in a linear concentration range of 1.0 ng mL^{-1}–100.0 ng mL^{-1}. The performance of this electrochemical immunosensor were carried out using different proteins.

1. Introduction

CEA is a crucial disease biomarker for lung cancer [1,2], colorectal, breast [3–5], pancreas, liver and ovarian cancer. It is recognised as an effective prognostic indicator for monitoring the stage of cancer in clinical assays. Meanwhile, the level of CEA in the blood should be minimum for healthy human whereas an abnormal level indicates the sign of cancer [6]. Therefore, an early diagnosis of biomarker is an important issue in improving the successful treatment of the disease and it leads to increase the survival of the patients [7–9]. Thus, it is required to produce better diagnostic device with rapid sensing capabilities. The most significant factors for diagnostic devices are high sensitivity, low cost, easy fabrication and prompt detection. In addition, it should possess high selectivity, specificity and reliability in the recognition of biomarkers [10–12]. Several quantitative methods have been used on which includes colorimetry [13,14], electrochemiluminescence [15–17], surface enhanced Raman spectroscopy [18], fluorescence [19] and electrochemical detection [20–22]. Among these methods, electrochemical method has been chosen because of simple operation, easy fabrication, high sensitivity and selectivity with portability. Electrochemical biosensor comprises of biological layer (antibody), which can chemically interact with the substance to be examined and a transducer system, which can identify the interaction and transform the biological signals into an electrical signal. Antibody based electrochemical biosensor detects the biomolecules through the interaction between an antibody and an antigen, have shown the great potential in biosensors applications [23–25].

In recent years, low-cost, efficient, reliable and rapid monitoring point-of-care devices has bought attention to the scientific community [26–28]. Cellulose paper has been chosen as substrate for electrochemical biosensor because of it has good physical and chemical properties. It is cheap, thin, flexible, biodegradable, easy disposable and it is composed of porous material made of homopolymer (1, 4- β glucopyranose) through acetyl bonds [29]. Paper-based electrochemical biosensors are fabricated by patterning the paper into hydrophilic channel enclosed by hydrophobic barriers. Various methods have been adopted

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to fabricate paper-based devices such as wax pattering [30], polydimethylsiloxane (PDMS) patterning [31], photolithography [32,33], plasma treatment [34] and screen-printing [35,36]. Among them, screen-printing technique has fascinated more due to its simple processing steps and bulk production. It adopts layer-by-layer deposition of conductive ink upon the substrate surface, through the mesh of desired geometry of the sensor. Therefore, the viscous conductive ink can be elated in the permeable surface through capillary action to form hydrophilic channels. In this work, we use self-made conductive gold ink for the deposition of working, counter electrode and commercial silver ink is used for the reference electrode. Self-made conductive gold ink is deposited on the paper substrate using self-assembly monolayer (SAM) technique. It uses the principle of chemical adsorption for immobilization of antibody onto the substrate. A SAM was on the solid matrix surface through chemical bonds such as Au-S, Si-OH etc. [37,38]. Meanwhile, numerous paper-based biosensors for biological analyses were demonstrated such as uric acid [39], human chorionic gonadotropin [40], human papillomavirus [41] and CEA [42]. For the rapid label-free detection of biomarkers, organic synthesis based material functionalization is implemented for electrochemical sensing of CEA. In this work, we fabricate a paper based screen-printed gold electrode (P-SPGE) with quinone centred compound.

Quinones are one of the organic compounds, which may be named as diketones. It has a substantial remark on various biological, pharmaceutical and chemosensors applications [43–45]. Quinones are exhibiting good redox active cycles with their semiquinone radicals, leading to the formation of reactive oxygen species. The redox responses of the quinone while interacting with biological compounds have been recorded using electrochemical methods. The fast response detection of CEA is measured using DPV. It is the most used electrochemical methods among other methods [46,47]. The interaction between the antigen, antibody takes place on the surface of the electrochemical biosensor can be measured in the form of current change in amperes [48,49].

This study aims to acquire a novel, selective, sensitive and reproducible electrochemical immunosensor to detect the CEA using **R1**/anti-CEA on P-SPGE. **R1** was prepared to use as self-assembled monolayer (SAM) on the P-SPGE surface. The amine group in the **R1** was activated using the crosslinking chemistry of N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC)/N-Hydroxysuccinimide (NHS). After that, the SAM layer was utilised for the covalent bonding between the carboxylic groups of anti-CEA with activated amine groups of **R1.** The Quinone moieties were flexible and having good redox-active property to detect the CEA using DPV measurements. The analytical studies of CEA detection was performed for quantitative detection and low detection limit was obtained. The performance of P-SPGE was compared with commercial screen-printed Au electrode. To confirm the reliability of results, the specificity of the electrochemical immunosensor was calculated using different proteins. To the best our knowledge, this kind of simple Quinone based electrochemical immunosensors renders the excellent results towards the highly sensitive detection of CEA.

2. Materials and methods

2.1. Chemicals

Whatman® grade 1 chromatography paper, monoclonal carcinoembryo antibody, carcinoembryo antigen, chloroauric acid ($HAuCl_4{\cdot}3H_2O$), sodium borohydride ($NaBH_4$), poly (N-vinylpyrolidone) and Silver ink were purchased from sigma-aldrich, India. Lawsone, 2-Mercaptoethylamine, Hydrochloride. Screen-printed gold working electrode and a counter electrode with a diameter of 4.0 mm^2 from Dropsens DRP-220AT, Spain, and the reference electrode was silver/silver chloride (Ag/AgCl). The specific potentiostat connector was an Autolab potentiostat/galvanostat model PGSTAT 30 (EcoChemic, Utrecht, Netherlands) and NOVA software (version 2.0) was utilised as the interface. N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), N-Hydroxysuccinimide (NHS) and sodium borohydride were purchased from sigma-aldrich, India. Sodium phosphate dibasic and sodium phosphate monobasic were purchased from Merck. All the chemicals and spectroscopic grade solvents used in the present study were of high purity analytical grade (Aldrich and Merck, India) and were used as received. Working solutions of anti-CEA and CEA were prepared by dilution in phosphate buffer solution (PBS). PBS solution (10 mM, pH 7.4) was prepared by dissolving of sodium phosphate dibasic and sodium phosphate monobasic in deionized water.

2.2. Instrumentation

Nuclear magnetic resonance spectra were recorded in DMSO-d_6 (Bruker, ^1H NMR 400 MHz, ^{13}C NMR 100 MHz). The ^1H NMR spectra data was expressed in the form: chemical shift in units of ppm (normalized integration, multiplicity, and the value of J in Hz). All electrochemical measurements were performed using a CHI Model 842 B Electrochemical Workstation. Atomic force microscopy (AFM) experiments were obtained with Veeco XE-100E and data were analysed with XEI imaging software. Scanning electron microscopy (SEM) images were obtained and analysed with JEOL SEM 7001-F. UV–vis absorption spectra were measured using JASCO V-550 spectrophotometer. High-resolution transmission electron microscopy (HRTEM) images were taken from JEOL JEM 2100, USA operating at 200 kV. Particle size distribution measurement was measured by a Nano ZS ZEN3600, Malvern Instruments Ltd. Dynamic light scattering (DLS) measurement was carried out with Nano ZS ZEN3600. Viscosity measurement was showed with Ubbelohde viscometer.

2.3. Preparation of AuNP ink

With reference to the article [50], AuNP ink were synthesised with poly-N-vinylpyrolidone (PVP) protection. First, AuNPs were produced through chemical reduction of $HAuCl_4$ via $NaBH_4$ addition. To prevent the agglomeration of AuNP, PVP acts as a capping agent. Next, 0.015 g of PVP is added to 20 mL of $HAuCl_4{\cdot}3H_2O$ and the solution was stirred for 20 mins. The solution of $NaBH_4$ (0.05 mL) was prepared and placed in freezer until it reached <5 °C. The $NaBH_4$ solution was added and stirred (4 h) to the nanoparticle solution and color change (bright yellow to dark purple) was observed and centrifuged (5000 rpm) for 30 mins. Finally, the pellet of AuNP protected with PVP is dispersed in isopropyl alcohol (70%) and DI water (30%) through ultra-sonication. Now the AuNP ink was ready for screen-printing. The synthetic scheme of AuNP ink preparation was shown in Fig. 1. Characterization of AuNP ink were illustrated in supplementary file.

2.4. Synthesis of R1

To a suspension of lawsone (500 mg, 2.9 mmol) in ethanol (10 mL) was added 2-Mercaptoethylamine (244 mg, 3.1 mmol) and the mixture was kept at room temperature for 10 mins. After addition of 4-aminobenzaldehyde (417 mg, 3.4 mmol), the reaction mixture was left stirring in the dark for 24 h. The orange solid was filtered, washed with ethanol and water, dried and recrystallized from methanol (Scheme 1).