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Full Paper

A Flexible Paper-based Electrochemical Immunosensor Towards Detection of Carbohydrate Antigen 15-3

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Abstract- In this paper, we developed a simple and disposable electrochemical paper-based immunosensor (e-PI) based on manual screen printing method via patterned sticker label film on paper. The proposed electrochemical sensors provide the opportunity for economical single-use analysis of biological samples. The e-PI was constructed with hydrophobic glossy paper layers on a wax paper substrate to define three-electrode system. A carbon ink was used for construction of e-PI which composed of the graphite powder and cellulose acetate. The stability of the e-PI was evaluated using electrochemical methods. Carbohydrate antigen 15-3 (CA15-3) antibodies to the target CA15-3 antigen were immobilized on gold-modified graphite electrodes on the e-PI. Electrochemical properties of e-PI were investigated by cyclic voltammetry, differential pulse voltammetry and electrochemical impedance spectroscopy. The fabricated e-PI offers a vast linear range from 0.5 to 200 U mL^{-1} with a low limit of detection 0.15 U mL^{-1}. Furthermore, the fabricated e-PI has acceptable stability, accuracy and high sensitivity.

Keywords- Paper-based sensor; CA15-3 biomarker; Immunosensor; Graphite ink; Biosensor

1. INTRODUCTION

Development of simple, low-cost and disposable electrochemical detection system is extremely important and beneficial in medical diagnosis and environmental analysis for

quantitative detection of various analytes, including cancer biomarkers [1,2], cells [3], pharmaceutical drugs [4–7], bacteria [8] and metal ions [9]. In this direct, different electrochemical platform including carbon paste electrode [10], ITO [11], glassy carbon electrode [12–15] and paper-based electrode [16,17] have been developed. Among these sensing platforms, paper-based electrodes attracted remarkable interest due to their excellent properties such as flexibility, low-cost, accessibility, facile miniaturization and compatibility [18]. To construct paper-based electrodes, it is necessary to develop simple and reliable methods via prototype manufacturing processes. So far, different methods have been developed to manufacture electrochemical paper-based electrodes such as wax printing [19], laser treatment [20], photolithography [21], plasma etching [22], and flexography printing [23]. The harmful reagents to create hydrophilic areas limit the use of the mentioned technique. Therefore, the development of reagent-free and prototype manufacturing processes is important for the development of paper-based detection systems. Recently, we have reported a low-cost and simple technique for the prototype manufacturing paper-based electrode via patterned sticker as a hydrophobic boundary on the paper with lab-made carbon ink [24]. The paper-based electrode based on graphite inks shows low background currents and broad electrochemical windows as well as compatibility with various types of modifiers [25-27].

Cancer biomarkers are biological molecules in body fluids which can provide information about cancer in the body [28]. Therefore, the fast and sensitive detection of biomarkers in body fluids provides key information for early diagnosis and follow-up of diseases. Since, the most biomarkers are present in low concentrations in body fluids, so sensitive detection systems played a key role in the clinical diagnosis of diseases [29]. CA153 has routinely been applied as a standard biomarker in breast cancer patients for the tracking therapy and early diagnosis of the disease [30]. Thus, the development of a simple, reliable and accessible detection method is of great significance to the fast detection of low amounts CA153 in body fluids. So far, the common detection method for CA153 biomarker includes chemiluminescence [31], fluorescence [32] and electrochemical biosensors [33]. However, the most of these methods require expensive apparatus and not ideal for routine analysis. Among these methods, electrochemical biosensors are a powerful detection method due to the intrinsic properties such as sensitivity, low-cost, portability and ease of use [34–39]. The present work reports disposable and cheap paper-based sensor with simple and rapid fabrication, which can use for label-free detection CA153 biomarker. CA153 biomarker concentration was determined by changing the peak currents of the probe at the proposed paper-based immunosensor. Furthermore, the e-PI was successfully applied for the detection of CA153 biomarker in serum samples to confirm the practical feasibility of the fabricated paper-based platform. The proposed e-PI showed acceptable electrochemical characteristic as a paper based sensor toward detection of CA153 biomarker.

2. EXPERIMENTAL

2.1. Chemicals and apparatus

Graphite powder, potassium chloride, HAuCl4, potassium ferrocyanide, ethanol, phosphate salt and hydrochloric acid are from Merck. Poly (acrylic acid), cellulose acetate and ethanolamine are from Sigma Aldrich. Self-adhesive paper was purchased from Taha label, Iran. The morphology of the electrodes was investigated by scanning electron microscopy (TESCAN, Czech Republic). Electrochemical experiments were conducted with an Autolab PGSTAT101 potentiostat/galvanostat (Eco Chemie, Netherlands).

2.2. Preparation of paper-based electrode

The electrodes were prepared according to our previous work [24]. Briefly, the patterned sticker as a hydrophobic boundary was prepared using a laser cutter (Figure 1a and b). Next, e-PIs were prepared with patterned sticker on the paper via screen printing of prepared carbon ink (Figure 1c). A carbon ink was constructed by mixing 0.4 g graphite powder and 0.1 g cellulose acetate solution (10% w/w in acetone and cyclohexanone). In the next step, the reference electrode of e-PI was prepared with silver-silver chloride. The detail of this step was discussed in our previous paper [40].

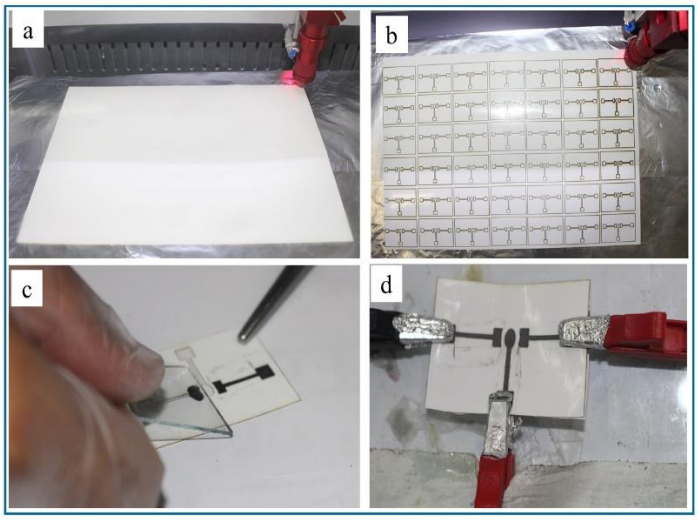

Figure 1. (a-d) Different steps of the e-PI fabrication

2.3. Fabrication of electrochemical paper-based immunosensor

The working electrode (WE) of e-PI was modified with gold nanoparticles (Au/e-PI) by cyclic voltammetry in the presence of 0.4 mM HAuCl4 in the range of −200 to 1400 mV at the scan rate of 40 mV s^{-1}. For immobilization of anti-CA15-3 (Ab) on the e-PI, 8 µL of CA15-3 antibody (50 ppm) was casted on the Au/e-PI for 80 min and then the electrodes were rinsed with buffer solution (Ab/Au/e-PI). After that, the empty spaces between anti-CA15-3 were

covered with 1.0 mM MCH solution (for 30 min). The Ab/Au/e-PI was used to detect of CA15-3 antigen. Figure 2 shows the preparation steps of Ab/Au/e-PI.

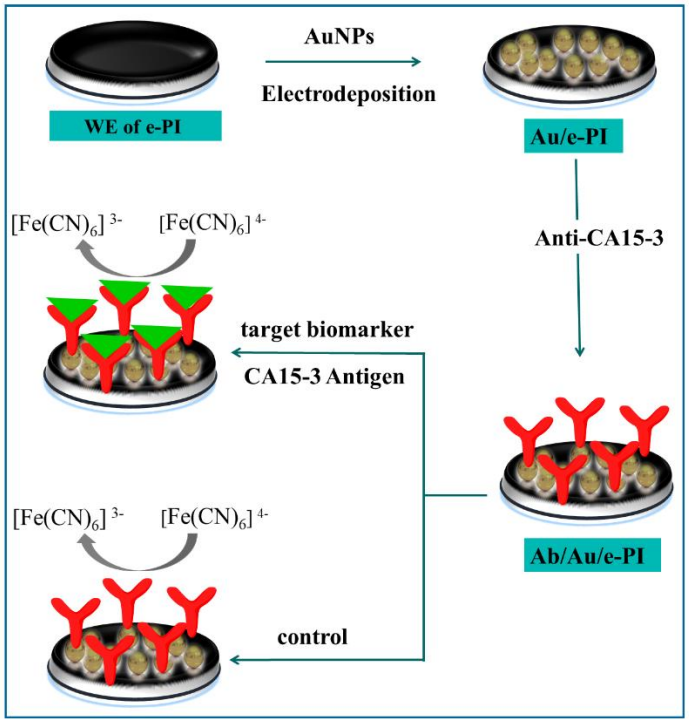

Figure 2. Schematic exhibition of the fabrication process of the immunosensor

3. RESULTS AND DISCUSSION

3.1. Characterization of the e-PI

The structure and morphology of the working electrode of the e-PI and Au/e-PI were investigated by SEM (Figure 3). The thickness of the carbon layer on the paper is 40.8±1.1 μm (Figure 3a), which is dependent on sticker thickness. Figure 3b and c show graphite particles were homogeneously decorated with Au nanoparticles.

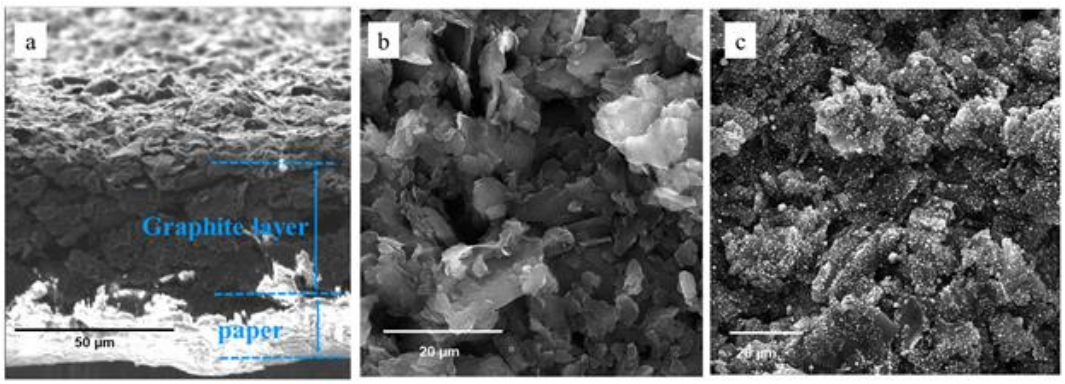

Figure 3. SEM images of cross-section (a) and top view of e-PI (b) and Au/e-PI (c)

The fabricated e-PIs were investigated using CV and EIS by with 1.0 mM $[Fe(CN)_6]^{3-}$ /$[Fe(CN)_6]^{4-}$ probe (Figure 4). CVs of the e-PIs and shows a couple of redox peaks with ΔEp of 98 mV (Figure 4a). As can be seen, in the presence of Au NPs on the WE (Au/e-PI), the ΔEp was decreased and peak currents were increased due to the catalytic effect of Au nanoparticles. In the EIS studies (Figure 4b), the R_{ct} of Au/e-PI was decreased compared to R_{ct} obtained for e-PI due to the enhancement of the electron transfer in the presence of Au NPs on the WE (Au/e-PI) [41–44]. The stability of fabricated e-PIs and Au/e-PI were evaluated by twelve successive CV in the in the presence of 1.0 mM $[Fe(CN)_6]^{3-/4-}$, and the results are given in Figure 4c and d. No obvious changes in E_p and I_p obtained confirm that fabricated e-PIs and Au/e-PI are stable and reproducible.

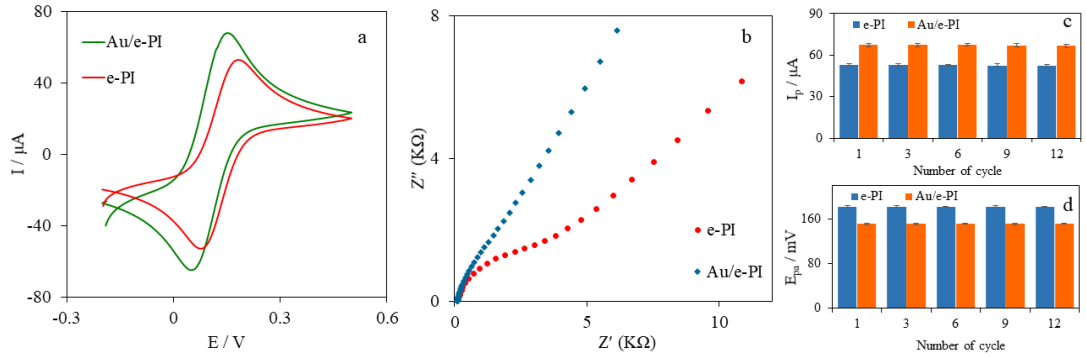

Figure 4. CVs (a) and Nyquist plots (b) of e-PI and Au/e-PI in 0.1 M KCl and 1.0 mM $[Fe(CN)_6]^{3-/4-}$ at a scan rate of 100 mV s^{-1}. Effect of successive CVs on the I_p (c) and E_{pa} (d)

3.2. Electrochemical behaviors of Ab/Au/e-PI

CV was applied to characterize each step of immunosensor fabrication, the possibility of sensing platform blocking on the various step of immunosensor fabrication. As illustrated in Figure 2, the first step of immunosensor construction is the immobilization of anti-CA15-3 on the Au/e-PI. The detection of CA15-3 antigen (Ag) is based on the antibody-antigen complex between the CA15-3 antigen in the solution and the CA15-3 antibody on the Ab/Au/e-PI. Figure 4 shows the CVs (Figure 5a) of Au/e-PI, Ab/Au/e-PI and Ag/Ab/Au/e-PI in the presence of 1.0 mM $[Fe(CN)_6]^{3-/4-}$. After immobilization of CA15-3 antibody on the Au/e-PI surface (Ab/Au/e-PI), CV peak current was decreased (Figure 5a and b) due to the breaking of electron transfer between the probe in solution and the Ab/Au/e-PI with the formed of CA15-3 antibody as blocking layers on the Ab/Au/e-PI. Also, after capturing of CA15-3 antigen using Ab/Au/e-PI (Ag/Ab/Au/e-PI) the CV peak current decreased due to the formation of CA15-3 antibody-CA15-3 antigen complex (Figure 5a and b). This blocking layer act as the interfacial electron-transfer blocking layer on the Ag/Ab/Au/e-PI. These results confirm that the immobilization of CA15-3 antibodies on the Au/e-PI and formation of CA15-3 antibody-CA15-3 antigen complex on the Ag/Ab/Au/e-PI surface.

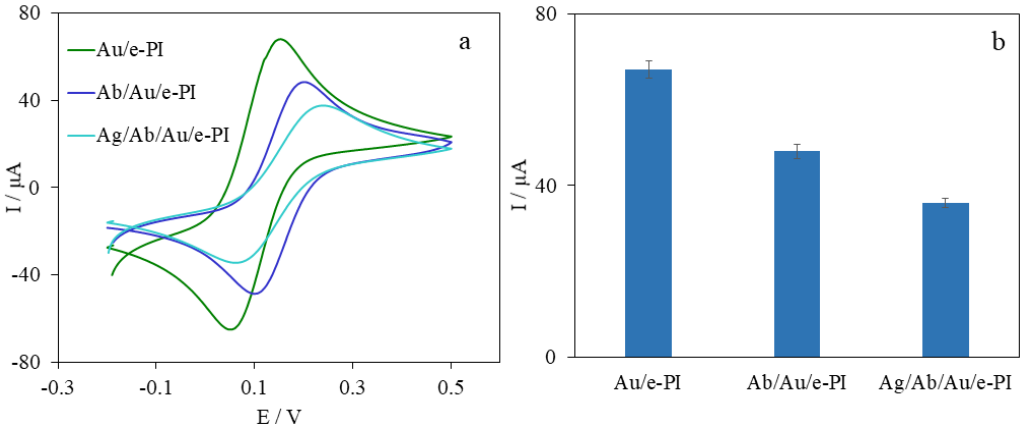

Figure 5. (a) CVs of Au/e-PI, Ab/Au/e-PI and Ag/Ab/Au/e-PI in 0.1 M KCl and 1.0 mM $[Fe(CN)_6]^{3-/4-}$ at a scan rate of 100 mV s^{-1}, (b) The CV peak currents of Au/e-PI, Ab/Au/e-PI and Ag/Ab/Au/e-PI

3.2. Optimization of experimental conditions

In order to the best performance of Ab/Au/e-PI, the affecting parameters on the immunosensor response were investigated. The incubation time of CA15-3 antibody on the Au/e-PI was evaluated in the different time. Based on the obtained results (Figure 6a), the DPV currents decrease with increasing incubation time of CA15-3 antibody and reaches to steady peak current after 80 min, therefore, 80 min was selected for Au/e-PI fabrication. Also, the effect of the different concentration of antibody was evaluated from 30 to 60 ppm (Figure 6b). The DPVs peak currents indicated that the Au/e-PI displayed an optimum response at 50 ppm concentration of CA15-3 antibody. Moreover, the incubation time of CA15-3 antigen on the Ab/Au/e-PI was evaluated (Figure 6c). The DPVs peak currents decreases with the elevated incubation time of CA15-3 antigen and then increase when the concentration oversteps the 150 min. Thus, the 150 min is selected as the optimized incubation time.

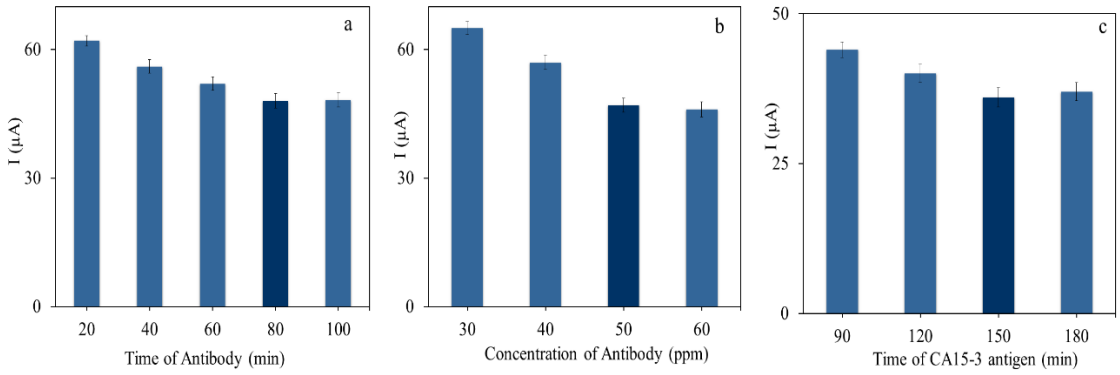

Figure 6. Optimization of: (a) incubation time of CA15-3 antibody (b) the concentration of CA15-3 antibody, (c) incubation time of CA15-3 antigen. The error bars represent RSD from five measurements

3.3. Analytical characteristics of Ab/Au/e-PI for CA15-3 antigen detection

The limit of detection and linear range of the Ab/Au/e-PI for CA15-3 antigen detection were investigated by DPV technique. The DPV peak currents decreases along with the increased concentration of CA15-3 antigen in the range of 0.5 to 200 U mL^{-1} (Figure 7a). The good linear correlation was observed between the DPV peak currents and the logarithm of CA15-3 antigen concentrations (Figure 7b). The LOD of Ab/Au/e-PI for CA15-3 antigen detection was 0.15 U mL^{-1} based on the signal-to-noise ratio of 3σ.

The selectivity of the Ab/Au/e-PI was evaluated for detection of 20 U mL^{-1} CA15-3 antigen in the presence of NSE, CEA and TNF-α biomarkers. Figure 7c shows the responses of Ab/Au/e-PI obtained for CA15-3 antigen in the presence of mentioned biomarkers, which shows negligible change in the presence of different biomarkers. Thus, the Ab/Au/e-PI has a good specificity for measurements of CA15-3 antigen.

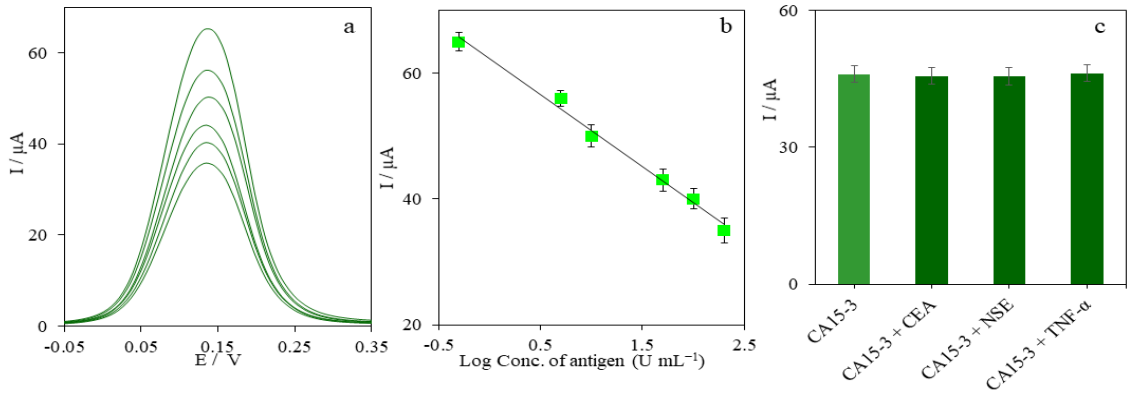

Figure 7. (a) DPV responses of the Ab/Au/e-PI for the detection of different concentrations of CA15-3 antigen (0.5 to 200 U mL^{-1}) in 0.1 M KCl and 1.0 mM $[Fe(CN)_6]^{3-/4-}$. (b) The calibration curve and (c) The selectivity of the Ab/Au/e-PI for detection of 20 U mL^{-1} in the presence of CEA, NSE and TNF-α

3.4. The repeatability and stability of Ab/Au/e-PI

The reproducibility of Ab/Au/e-PI was evaluated by the measurements of 20 U mL^{-1} CA15-3 antigen by six Ab/Au/e-PI fabricated with the same method. The RSD of six measurements was less than 4.2% for different Ab/Au/e-PI. Thus, the proposed immunosensor has an acceptable reproducibility. To investigated of the stability of Ab/Au/e-PI, the prepared sensors were stored at 4 °C. The response reduces 5.2% after 18 days, which indicated that the Ab/Au/e-PI had acceptable stability.

4. CONCLUSIONS

In summary, a paper-based immunosensor for detection of CA15-3 antigen was developed based on the simple and economic technique. The response of this immunosensor was based

on the changes of interfacial properties of sensing platform by interaction between the CA15-3 antigen in the solution and the anti-CA15-3 on the Ab/Au/e-PI. The fabricated Ab/Au/e-PI shows acceptable stability, reproducibility and specificity in measurements of CA15-3 antigen, detection limit as 0.15 U mL^{-1}, and a wide linear range from 0.5 to 200 U mL^{-1}.

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Conflicts of interest/Competing interests

The authors declare that they have no competing interests.

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