Graphene/Polyaniline Nanocomposite Modified Electrode for Biosensors

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ABSTRACT

A novel electrochemical biosensor has been developed for determination of neutrophilgelatinase-associated lipocalin(NGAL).The developed electrodes were fabricated by electrospraying of graphene/polyaniline (G/PANI) nanocomposites followed by electropoly-merization of aniline on the modified electrode. In this study, the factors affecting the electrode surface area and electrochemical sensitivity, such as aniline concentration, scan number of electropolymerization were investigated and optimized. The morphology of modified electrodes was characterized by scanning electron microscopy (SEM) and the electrochemical characterization was performed by cyclic voltammetry (CV). The NGAL antibody was immobilized on the modified electrode for detection of NGAL protein. This system was successfully used for the sensitive determination of NGAL at a concentration as low as 90 ng/ml. This novel biosensor might be an alternative tool for early stage diagnosis of acute kidney injury.

Keywords: Graphene, polyaniline, electropolymerization, NGAL

1. INTRODUCTION

Acute kidney injury (AKI) or acute renal failure (ARF) is a loss of kidney function within 2 hours to 2 days , increasing the risk factor to mortality and complication in patients after cardiac surgery [1, 2]. Thus, AKI diagnosis has been developed to increase the accuracy and sensitivity for treatment of patients. In the past, AKI was diagnosed by the determination of serum creatinine (SCr) [3]. Unfortunately, SCr is an unreliable indicator for AKI diagnosis because the concentration of creatinine is not significantly change until loss of at least 50% of kidney function [4]. Hence, it is necessary to search a new biomarker for AKI diagnosis. Neutrophil gelatinase-associated lipocalin (NGAL) has been focused. NGAL is a 25 kDa protein found in both human urine and blood. In general, NGAL concentration increases before SCr concentration increases [5, 6]. Therefore, NGAL is selected to be a useful biomarker for early diagnosis of AKI [7-10]. Various analytical techniques such as ELISA [11, 12] and immunoblotting have been used for the determination of NGAL [4]. Recently, an electrochemical based method has been developed and used as a highly sensitive biosensor for NGAL [13].

Electrochemical technique has been widely used in the determination of various substances, such as food contaminants, environmental pollutants and disease biomarkers. Working electrode is the most important part in the electrochemical analysis. For sensor applications, working electrode is usually designed to be a small size to make it portable; however, the tiny size of electrode limits the surface area and sensitivity for electrochemical detection. Thus, increasing the surface area of electrode is important for the improvement of electrochemical sensitivity. To solve this problem, various nanomaterials, such as carbon nanotubes (CNTs), carbon nanofibers (CNFs) and carbon nanodot (CNDs) have been used for electrode surface modification to increase both electrode surface area and electrochemical sensitivity. Recently, graphene (G) a two dimensional crystalline allotrope of carbon has become an interesting material due to its large surface area, high electrical conductivity, high stability, and low cost [14-16]. To prevent the agglomeration of G, conducting polymer, such as polyaniline (PANI) has been used along with G for electrode modification. PANI is one of the conducting polymers attracting a great attention due to its high conductivity, reversible redox reaction, easy synthesis and biocompatibility [17, 18].

Here, the working electrode is fabricated by electrospraying of G/PANI nanocomposite and follow by electropolymerization of aniline for sensitive determination of NGAL.

2. MATERIAL AND METHODS

2.1 Chemicals and materials

Graphene nanopowders were purchased from SkySpring Nanomaterials. Inc, (Houston, TX, USA). NGAL protein and capture NGAL antibody were purchased from Abcam Ltd., UK and used as received. Polyanilineemeraldine base (M_w = 65,000), (+)-camphor-10-sulfonic acid (CSA), Polystyrene (M_w = 180,000), potassium ferricyanide ($K_3[Fe(CN_6)]$), potassium ferrocyanide ($K_2[Fe(CN_6)]$), phosphate buffer saline (PBS, pH 7.2), 1-ethyl-3-(3-dimethyaminopropyl) carbodiimide (EDC), N-hydroxysuccinimde (NHS) were purchased from Sigma-Aldrich (St, Louis, MO, USA). Potassium chloride (KCl) was purchased from PFCL, ltd. (New Delhi, India). Chloroform, dichloromethane, N,N-dimethylformamide (DMF) were obtained from Carlo Erba Reagents (Milano, Italy). All solution were prepared in MilliQ water system (Millopore, USA, R ≥ 18.2 MΩ cm^{-1})

2.2 Apparatus

All electrochemical measurements were performed on a CHI 1240B electro-chemical analyzer (CHI Instruments, Inc., USA). A three of electrode system was used and a screen-printed carbon working electrode (4 mm in diameter) was modified with G/PVP/PANI. Electrospraying system was used for electrode modification and follow by electropolymerization of aniline monomer. A JSM-6400 field emission scanning electron microscope (Japan Electron Optics Laboratory Co., Ltd, Japan) was used for the electrode characterization.

2.3 Electrospraying of G/PANI nano-composites modified on the screen–printed carbon electrodes

For G/PANI nanocomposite solution, G nanopowder and PVP (2:2 mg) were dispersed in 1 mL DMF using an ultrasonicator for 24 h at room temperature. PANI (0.4 g) was doped with CSA (0.516 g) and dissolved in 15 mL chloroform. The solution of G/PVP and PANI were mixed together and 0.1% (v/v) PS was added into solution. An applied voltage of 7.5 kV, flow rate of 1.0 mL/h, distance of 5 cm, and 5 min electrospraying were used.

2.4 Electrochemical measurement

All the electroanalytical measurements were performed on a CHI 1240B electrochemical analyzer (CHI Instruments, Inc., USA). For the cyclic voltammetry, the potential was scan from -0.5 V to +1.0 V for electropolymerization of aniline monomer,-0.5 V to +0.1 V for ferri/ferrocyanide detection and -0.2 V to +0.6 V for NGAL detection.

2.5 Functionalization of modified electrode

The G/PANI modified electrodes were functionalized with NGAL capture antibody by using EDC/NHS reaction. In this study, a mixture solution of EDC/NHS (0.2/0.2 M) and NGAL antibody (360 µg/ml) were dropped on the working electrode surface and incubated for 3 h at room temperature in a dark area. Then, the modified electrodes were washed using PBS buffer and MilliQ water to eliminate the unbounded antibody.

3. RESULTS AND DISCUSSION

3.1 System optimization

In this study, a novel electrochemical biosensor has been developed for the determination of NGAL. The electrodes were fabricated by electrospraying of G/PVP/PANI nanocomposite for increased the surface of electrode and follow by electropolymerization of aniline for increased the electrochemical conductivity and increased amino group (NH_2) on the surface for antibody functionalization. Schematic diagram of G/PANI modified electrode for NGAL detection is shown in Fig 1.

The electrospraying parameters have been optimized as shown in the previous report [18]. In this study, the other factors affecting the modified electrode surface area and electrochemical sensitivity, such as aniline monomer concentration, scan number of electropolymerization, electrode configuration were investigated and optimized.

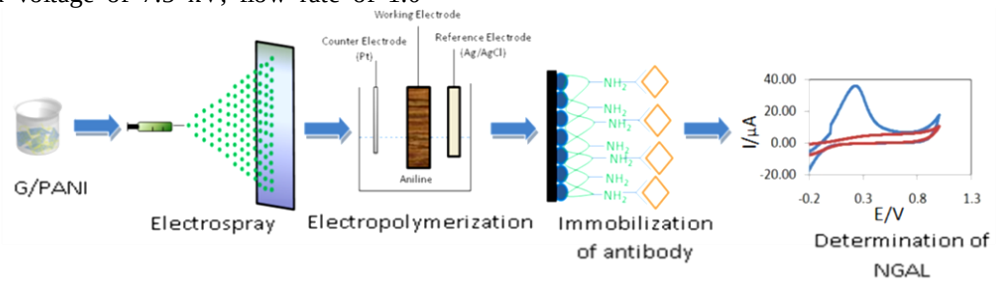

Fig 1. Schematic diagram of G/PANI modified electrode for NGAL detection.

Aniline monomer was polymerized on the surface of G/PANI nanodroplet modified electrode. The thickness of polymer was control by number of scan for aniline electropolymerization (2, 4, 6, 8, 10). The results from cyclic voltammetric measurement using ferri/ferrocyanide as a standard couple [$Fe(CN_6)^{3-/4-}$] show the effect of scan number on the electrochemical sensitivity of modified electrode (Fig 2). The anodic peak current gradually increases when the number of scan increases from 2 to 10. However, the well-defined and symmetric cyclic voltammogram is obtained at a scan number of 2 and 4 only (Fig 2a) indicating that the electron transfer process is mainly controlled by diffusion. For the other scan numbers (6, 8, 10), the adsorption probably takes place on the electrode surface leading to asymmetric anodic peak current at a high potential range (0.7-0.9V). Thus, a scan number of 4 is selected for electropolymerization of aniline in this study.

To investigate the influence of aniline concentration on the electrochemical sensitivity of modified electrode, different concentrations of aniline ranging from 0.01-0.10 M were used for the electropolymerization. The results obtained from cyclic voltammetric measurement are shown in Fig 3. The increase of aniline concentration significantly enhance the anodic peak current of 1.0 mM standard $[Fe(CN_6)^{3-/4-}$, leading to increased the electrochemical sensitivity of modified electrode. Therefore, 0.10 M of aniline, which provide the highest anodic peak current was chosen for the electropolymerization in further experiments.

3.2 Characterization of G/PANI modified electrode

The morphology of the modified electrode was characterized by scanning electron microscopy (SEM) as shown in Fig 4a. The homogeneous and porous network of electropolymerized aniline is observed on the modified electrode surface.

The electrochemical characteristics of the modified electrode were monitored by cyclic voltammetry, using a standard $[Fe(CN)_6]^{3-/4-}$ redox couple. As shown in Fig.4b, both anodic and cathodic currents measured on electropolymerized aniline modified G/PANI electrode are much higher than the current responses measured on an unmodified electrode about 4 times. The increasing of current responses showed the enhanced electrochemical sensitivity of this modified electrode. Then, the modified electrode was applied for the determination of NGAL protein by using cyclic voltammetry.

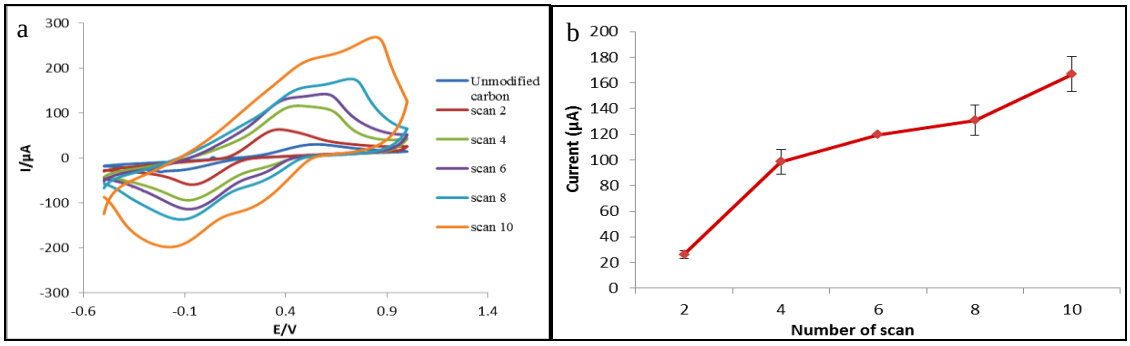

Fig 2. (a) Cyclic voltammograms of 1.0 mM $[Fe(CN)_6]^{3-/4-}$ measured on electropolymerized G/PANI modified electrode at scan number of 2, 4, 6, 8, 10 and (b) the anodic peak current (i_{pa}) obtained from the cyclic voltammogram in Fig 2a.

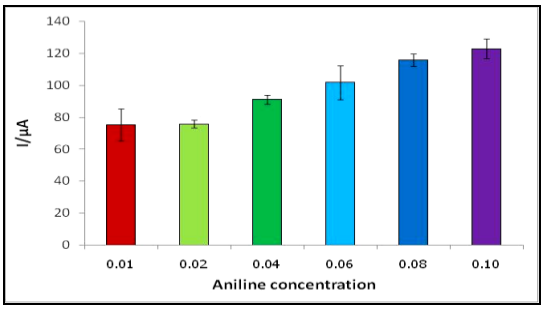

Fig 3. Anodic peak currents obtained from cyclic votammogram of 1.0 mM $[Fe(CN_6)^{3-/4-}$ measured on electropolymerized G/PANI modified electrode using different concentration of aniline.

3.3 Electrochemical response of NGAL

To study the electrochemical response of NGAL using cyclic voltammetry, the NGAL antibody was immobilized on the modified electrode via covalent bonding. The electrochemical determination of NGAL on a modified electrode was carried out in 0.2 M PBS at a pH of 7.2 as shown in Fig 5. NGAL protein is sensitively detected on the modified electrode (b) and the cyclic voltammetric signal substantially increases compare to the background signal (a). The lowest concentration of NGAL protein is found to be 90 ng/mL.

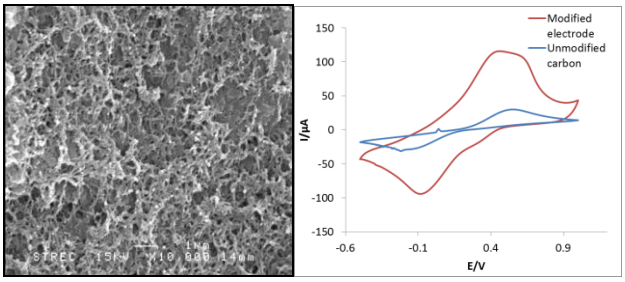

Fig 4. (a) an SEM image of electropolymerized G/PANI modified electrode and (b) cyclic voltammograms of 1.0 mM standard $[Fe(CN)_6]^{3-/4-}$ measured on a modified electrode compare with an unmodified electrode.

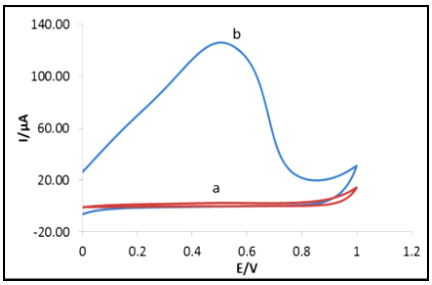

Fig 5. Cyclic votammograms measured on the modified electrode in the absence (a) and presence (b) of 90 ng/ml of NGAL in 0.2 M PBS at a scan rate of 100 mV/s.

4. CONCLUSION

In summary, electropolymerized aniline on G/PANI nanodroplet modified electrode was successfully prepared. The modified electrode showed the homogeneous and porous network surface and exhibited the significantly enhanced electrochemical sensitivity compared to an unmodified electrode. Eventually, this system was applied for the sensitive determination of NGAL protein and the NGAL concentration as low as 90 ng/mL was successfully detected. This system will be applied for the detection of NGAL in real biological samples and it might be an alternative tool for early stage diagnosis of acute kidney injury.

5. ACKNOWLEDMENT

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