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AuNPs/CNOs/SWCNTs/chitosan-nanocomposite modified electrochemical sensor for the label-free detection of carcinoembryonic antigen

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ABSTRACT

In this work, a nanocomposite of gold nanoparticles (AuNPs), carbon nano-onions (CNOs), single-walled carbon nanotubes (SWCNTs) and chitosan (CS) (AuNPs/CNOs/SWCNTs/CS) was prepared for the development of highly sensitive electrochemical immunosensor for the detection of carcinoembryonic antigen (CEA), clinical tumor marker. Firstly, layer-by-layer fabrication of the CEA-immunosensors was studied using cyclic voltammetry (CV) and square wave voltammetry (SWV). By combining the advantages of large surface area and electronic properties of AuNPs, CNOs, SWCNTs, and film forming properties of CS, AuNPs/CNOs/SWCNTs/CS-nanocomposite-modified glassy carbon electrode showed a 200% increase in effective surface area and electronic conductivity. The calibration plot gave a negative linear relationship between log[concentration] of CEA and electrical current with a correlation coefficient of 0.9875. The CEA-immunosensor demonstrated a wide linear detection range of 100 fg mL^{-1} to 400 ng mL^{-1} with a low detection limit of 100 fg mL^{-1}. In addition to high sensitivity, reproducibility and large stability, CEA-immunosensor provided an excellent selectivity and resistant-to-interference in the presence of other antigens in serum and hence a potential to be used with real samples.

1. Introduction

Carcinoembryonic antigen (CEA), a type of acidic glycoprotein with a molecular weight of about 200 kDa, is known as one of the most important clinical tumor biomarkers (Adam et al., 2003). It is reported that an increase in the concentration of CEA in adult plasma may be an early indication of a number of cancerous diseases including colon tumors, breast tumors, ovarian carcinoma, colorectal cancer and cystadenocarcinoma (Hernández et al., 2002; Eppler et al., 2002; Prete et al., 2005; Limbut et al., 2006; Duffy et al., 2003; Naghibalhossaini and Ebadi, 2006). Since raised level of plasma CEA may signify the presence of a tumor and indication of possible disease (Li et al., 2015), early detection of CEA, albeit at low concentrations is an important step for controlling, effective treatment (Ahmed et al., 2016), monitoring and screening disease recurrence (Kulasingam and Diamandis, 2008; Sun et al., 2013).

Immunoassay, that uses the principle of specific affinity between antibody and corresponding antigen, has become one of the main analytical methods in clinical examinations and biochemical analyses (Nourani et al., 2013; Lim et al., 2014; Lim and Ahmed, 2015, 2016a, 2016b; Rizwan et al., 2017) due to high specificity and sensitivity of this technique. In recent years, various diagnostic methods, such as radioimmunoassay (Behera et al., 1997), enzyme immunoassay (Pina et al., 2001), chemiluminescence immunoassay (Lin et al., 2004), fluoroimmunoassay (Yuan et al., 2001), piezoelectric crystal based immunosensors (Shen et al., 2005), flow injection system based immunosensors (Wu et al., 2006), and surface plasmon resonance based immunosensors (Li et al., 2015) have been developed and used for analysis and detection of CEA. However, methods these have the drawbacks of using potentially dangerous reagent and thus radiation hazards; requiring qualified personnel, sophisticated instrumentation, expensive devices, complicated operation process; and being less sensitive and selective. These disadvantages greatly limit their practical application especially for in-situ and routine analysis. Thus, alternative approaches are required to curb these drawbacks (Huang et al., 2010).

Currently a few sandwich-type immunosensors have also been fabricated (Feng et al., 2016; Tian et al., 2016) for analysis and detection of CEA. However, in comparison to sandwich-type immunosensors, label-free format acquires many advantages including simple and straightforward fabrication, easy-handling, label-free, use of lesser chemicals and reagents, small analytes, less overall time and economical (Wu et al., 2013). To overcome the shortcomings of conventional assays and existing immunosensors, various efforts and approaches are being carried out to construct highly sensitive, reliable, fast, selective and economical CEA-immunosensors.

Among them, label-free electrochemical methods have received

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considerable attention because of their high sensitivity, excellent selectivity, fast analysis, simple pretreatment, small analyte volume, simple instrumentation with minimal manipulation, low cost, rapid response and simple operation (Goyal et al., 2007; Huang et al., 2010; Ahmed et al., 2014, 2016; Lim et al., 2014; Lim and Ahmed, 2015, 2016a, 2016b; Rizwan et al., 2017) and being use in fabrication of label-free CEA-immunosensors (Huang et al., 2010). Electroanalytical chemistry also plays a very important role in pharmaceutical drug screening analysis (Gupta et al., 2011a) and the protection of our environment (Gupta et al., 2011b). Therefore, various simple, inexpensive, sensitive and accurate analytical method has been developed for the analysis of pharmaceuticals, dug molecules and biological fluids such as cefixime (Jain et al., 2010), paracetamol (Goyal et al., 2010) and CH_3COO^- (Gupta et al., 2008). Whereas, highly selective, interference-resistant, sensitive, reproducible, and stable poly(vinyl chloride) (PVC)-membrane electrode based electrochemical sensors have also been developed to detect and monitor the concentration heavy, toxic and hazardous metals such as Pb^{2+} (Srivastava et al., 1995a; Gupta et al., 2006a; Jain et al., 2006), Cs^+ (Srivastava et al., 1995b), Zn^{2+} (Srivastava et al., 1996), Hg^{2+} (Gupta et al., 1996, 1997a, 2007a, 2013), UO_2^{2+} (Jain et al., 1997a, 1997b; Gupta et al., 1999), Ni^{2+} (Gupta et al., 1997b, 2000), Cd^{2+} (Gupta et al., 2002, 2014a), La^{3+} (Gupta et al., 2003), Cu^{2+} (Gupta et al., 2006b, 2012), Co^{2+} (Gupta et al., 2006c; Jain et al., 1997a, 1997b), Cr^{3+} (Gupta et al., 2006d), Al^{3+} (Gupta et al., 2007b), Fe^{3+} (Gupta et al., 2007c), as well as multi-walled carbon nanotubes and 1-n-butyl-3-methylimidazolium tetrafluoroborate based carbon paste electrode for Hg^{2+} detection (Khani et al., 2010) with high concentration range and low detection limit in body fluids, environment, industrial effluent, agriculture and domestic waste besides the fact that some heavy metal ions are essential to many organisms in small doses, high doses affect the ecosystem and human health. Electrochemical sensor has been also developed to monitor and detection the Hydroxylamine, Phenol and Sulfite in water and waste water samples (Gupta1 et al., 2015), aromatic amine in wastewater (Karthikeyan et al., 2012), CH3COO- in environment (Gupta et al., 2008) and potentiometric sensors for selective detection of N_3^- and NO_2^- (Prasad et al., 2004). On the other hand, recently fluorescent probe based sensor have also been developed for the sensitive and rapid detection of Mg^{2+} (Gupta et al., 2015a), Al^{3+} (Gupta et al., 2014a, 2015a, 2015b), acetate/fluoride ions (Gupta et al., 2015b).

Typically, an electrochemical immunosensor is composed of a biological element used for targets recognition and a transducer system that transforms any biorecognition event into a readable electrical signal (Lim and Ahmed, 2016b, 2015; Lim et al., 2014). Current trend shows that for this purpose various nanomaterials and nanocomposites are often incorporated to aid in biological recognition to increase target binding, amplify signal and consequently highly sensitive as well as selective detection (Khani et al., 2010; Gupta1 et al., 2015; Rizwan et al., 2017; Gao et al., 2016). Also, collaboration in nanotechnology and bioelectronics has uncovered new potential outcomes to scale down and fabricate highly sensitive label-free immunosensor by exploring the highly conducting nature and surface area of the nanoparticles and nanomaterials. When designing and fabricating a highly sensitive label-free electrochemical immunosensors, antibody immobilization and signal amplification are the most critical factors to consider (Sánchez et al., 2008). Many kinds of nanomaterial, including noble metal nanoparticles, carbon nanomaterials, semiconductor nanoparticles, metal oxide nanostructures, and nanocomposite, have been used to amplify electrochemical signal to improve the sensitivity of electrochemical immunosensors (Goyal et al., 2007; Pei et al., 2013). Many efforts have been done to improve the sensitivity of the label-free electrochemical CEA-immunosensors using various novel nanocomposites. For instance, Huang et al. (2010) used nano-Au/multi-walled carbon nanotubes–chitosans nanocomposite modified glassy carbon electrode for the detection of the CEA using differential pulse voltammetry (DPV) and achieved a limit of detection (LOD) 10 pg mL^{-1}. Gao et al. (2011) used chitosan-carbon nanotubes-gold nanoparticles nanocomposite to modify the glassy carbon electrode (GCE) for the sensitive detection of CEA using cyclic voltammetry (CV) and obtained a LOD of 40 pg mL^{-1}, whereas Huang et al. (2012) used a composite of gold nanoparticles (AuNPs), thionine and TiO_2-graphene to modify their GCE for the fabrication of highly sensitive CEA-immunosensors and achieved an LOD of 10 pg mL^{-1} using CV. In another current effort, Gao et al. (2016) used Nile blue A-reduced graphene oxide nanocomposites-GCE to fabricate label-free CEA-immunosensors and achieved an LOD of 450 fg mL^{-1}.

Nanocomposite of biopolymer, organic and inorganic nanomaterials is one of the key research fields of today's material science. It combines the physicochemical attributes of each components and provide novel composite materials with their improved features (Huang et al., 2010). Nanoparticles (NPs) and nanomaterials (NMs) exhibit unique chemical, physical and electronic properties that are different from their bulk counterparts, making them interesting for use with electrochemical sensors and biosensors (Gao et al., 2011, 2016; Lim et al., 2014; Lim and Ahmed, 2016b, 2015; Gupta1 et al., 2015; Rizwan et al., 2017). Amongst metal NPs, AuNPs are the most extensively used due to its novel and unique biocompatible, large surface to volume ratio and electronic properties (Lim et al., 2014; Gao et al., 2011). The advantageous properties of carbon materials make them ideal electrodes in electroanalytical chemistry. With the continuous and rapid progress of nanoscience and nanotechnology in material sciences, carbon nanomaterials, particularly single-walled carbon nanotube (SWCNTs) and carbon nano-onions (CNOs) have emerged recently as an excellent biocompatible nanomaterial with great structural, mechanical, and electronic properties. SWCNTs have been used in the fabrication of biosensors due to their unique and well-defined electrical and mechanical properties. SWNTs enhance electronic signal in electrochemical measurements by providing significantly larger surface area on the same site than a bare electrode due to high aspect ratio (Okuno et al., 2007; Goyal et al., 2010). In-addition to high conductive properties and surface area CNOs serve as linking layers between biomolecules and AuNPs of immunosensors resulting in signal amplification (Bartelmess and Giordani, 2014). Chitosan (CS) is a polysaccharide derived by deacetylation of chitin. CS has been widely used as an immobilization matrix for biofabrication due to excellent membrane-forming ability, high permeability towards water, good adhesion, biocompatibility, and high mechanical strength. The reactive amino and hydroxyl functional groups interact with the biorecognition element to provide biocompatible environment and long term functional stability (Huang et al., 2010).

Since the immobilization of biorecognition element onto the electrode surface is a crucial step for the construction of any electrochemical immunosensors, thus, searching for an effective and simple immobilization method is of considerable interest. Therefore, for the first time, our group prepared a nanocomposite of gold nanoparticles (AuNPs), carbon nano-onions (CNOs), single-walled carbon nanotubes (SWCNTs) and chitosan (CS) (AuNPs/CNOs/SWCNTs/CS) for the modification of GCE and development of highly sensitive label-free electrochemical immunosensor for the detection of carcinoembryonic antigen (CEA), clinical tumor marker using square wave voltammetry (SWV) as detection technique and $[Fe(CN)_6]^{3/4-}$ as mediator. By combining the advantages of large surface area and electronic properties of AuNPs, CNOs, SWCNTs, and film forming properties of CS, AuNPs/CNOs/SWCNTs/CS-nanocomposite-modified GCE showed a 200% increase in effective surface area and electronic conductivity. The CEA-immunosensor demonstrated a wide linear detection range of 100 fg mL^{-1} to 400 ng mL^{-1} with a low detection limit of 100 fg mL^{-1}. In addition to high sensitivity, reproducibility and large stability, CEA-immunosensor provided an excellent selectivity and resistant-to-interference in the presence of other antigens in serum and hence a potential to be used with real samples. This strategy provided an ecofriendly and