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3D label-free prostate specific antigen (PSA) immunosensor based on graphene–gold composites

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ABSTRACT

Highly sensitive and label-free detection of the prostate specific antigen (PSA) remains a challenge in the diagnosis of prostate cancer. Here, a novel three-dimensional (3D) electrochemical immunosensor capable of sensitive and label-free detection of PSA is reported. This unique immunosensor is equipped with a highly conductive graphene (GR)-based gold (Au) composite modified electrode. The GR-based Au composite is prepared using aerosol spray pyrolysis and the morphology of the composite is the shape of a crumpled GR ball decorated with Au nanoparticles. Unlike the previous research, this novel 3D immunosensor functions very well over a broad linear range of 0–10 ng/mL with a low detection limit of 0.59 ng/mL; furthermore, it exhibits a significantly increased electron transfer and high sensitivity toward PSA. The highest rate of current change with respect to the PSA concentration is 5 μA/(ng/mL). Satisfactory selectivity, reproducibility, and stability of the 3D immunosensor are also exhibited.

1. Introduction

Immunosensors based on antibody–antigen binding are one of the most widely used to detect disease related substances, which are known as biomarkers, in clinical diagnostics (Li et al., 2013). Due to the specific binding of an antibody to its corresponding antigen, antibodies are immobilized on the immunosensor surface in order to capture specific biomarkers (Mao et al., 2012). Among the numerous immunosensor species, the prostate specific antigen (PSA) for the specificity of prostate cancer markers has been widely used in prostate cancer screening, diagnosis, and treatment after monitoring (Huang et al., 2005; Qu et al., 2011). It is well known that the PSA concentration for a normal person ranges from 0 to 4 ng/mL (Qu et al., 2008; Yang et al., 2010).

There are two types of PSA immunosensors: sandwich-type immunosensors and label-free immunosensors. Sandwich-type immunosensors are primary composed of an antibody, secondary antibody, and antigen. This immunosensor can be prepared through the label protocol with the primary antibody immobilized on the solid surface and the specific antigen bound to the antibody site. The labeled secondary antibody can bind to the PSA antigen (Yang et al., 2010). In sandwich-type immunosensors, the labeled antibodies are used for signal amplification, and much attention has been paid to the development of materials for immobilizing more enzymes in order to increase the efficiency and sensitivity (Sun et al., 2013; Yang et al., 2011). In contrast, the label-free immunosensor can be prepared via direct detection of antibody–antigen interaction. This immunosensor has become a remarkable analytical tool for detection of biomolecules as a result of its simple preparation, fast detection, high sensitivity, and low cost (T. Li et al., 2011, R. Li et al., 2011).

In order to develop enhanced label-free immunosensors, graphene (GR)-based composites are of interest as electrode materials because GR has a high biocompatibility and fast electron transportation (Peng et al., 2012; Zhao et al., 2013). Akhavan et al. (2012) showed GR can effectively contribute to the development of ultra-high-sensitive electrochemical biosensors. T. Li et al. (2011) reported label-free electrochemical detection of PSA markers based on GR–cobalt hexacyanoferrate nanocomposites. They stated that the immunosensor using GR materials had advantages of high sensitivity, good selectivity, and stability. Mao et al. (2012) developed label-free electrochemical immunosensors for PSA based on GR–methylene blue nanocomposites. They reported that the amperometric signal decreased linearly with the PSA concentration (0.05–5.00 ng/mL) and that the immunosensor had a low limit of detection (0.013 ng/mL). The prepared PSA immunosensor was used in the analysis of PSA in serum samples with satisfactory results. As with the previous studies, the GR-based composite materials and nanomaterials garnered more attention as electrode materials due to the synergistic effect of two or more functional

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components in the potential sensor applications. Therefore, it is expected that a composite of GR and gold (Au) nanoparticles could exhibit enhanced sensitivities and simplify the assay system as a label-free PSA immunosensor. This is because GR can provide a better environment for electrochemical reactions for potential immunosensors, and Au nanoparticles are expected to enhance the conductivity, biocompatibility, and strong adsorption abilities (Huang et al., 2013; Liu et al., 2013; De et al., 2008, Kavosi et al., 2014). However, label-free immunosensors for PSA detection using GR–Au composites have not yet been reported.

In order to prepare label-free immunosensors fabricated with GR-based composites, previous studies have used a two-step or liquid phase reaction. However, these methods require numerous time consuming stages such as filtering, washing, and drying; they also require supporting materials that assist with the immobilization in order to prepare the composite materials. Because the morphology of the composite prepared via a liquid phase reaction is flat and two-dimensional (2D), 2D layered GR–Au sheets have a tendency to form aggregates and restack easily through π–π stacking and van der Waals attraction (Luo et al., 2011). Thus, it is expected that the 2D GR–Au sheets not only reduce their effective sensing ability but also compromise their properties, such as the accessible sensing surface area on the working electrode.

In our previous studies, we developed a crumpled GR-based composite exhibiting a three-dimensional (3D) morphology via aerosol spray pyrolysis (ASP) (Jang et al., 2012, 2013a, 2013b). We demonstrated that the crumpled GR-based composites had both a high free volume and a high compressive strength, and that they could be tightly packed without significantly reducing the accessible surface area, unlike the 2D GR sheets. The fabricated crumpled GR-based composites could also deliver much higher specific capacitances in the sensing performance. Therefore, crumpled GR–Au composites are expected to have a highly sensitive electrocatalytic activity of a PSA immunosensor compared with a 2D GR–Au composite. Furthermore, the Au nanoparticles in crumpled GR–Au composites can interact with many biomolecules (Fig. 1(a)). When crumpled GR–Au composites are used to fabricate label-free PSA immunosensors, 3D label-free PSA immunosensors can be prepared with high accessible surface areas that lead to enhanced biomolecule absorption and promote direct electron transfer between the materials and electrode surface.

In this study, 3D label-free PSA immunosensors based on crumpled GR–Au composites are introduced. The effects of the Au/GR ratio on the composite particle properties such as morphology and crystallinity are examined. Then, label-free immunosensors for the sensitive detection of PSA were prepared using the crumpled GR–Au composites modified with glassy carbon electrodes. The linear response range, low detection limit, selectivity, stability, and reproducibility were investigated for the detection of PSA as an immunosensor using cyclic voltammetry measurements.

2. Experimental

2.1. Preparation of crumpled GR–Au composites

Graphene oxide (GO) colloid was prepared via the oxidation of graphite powder (Alfa Aesar, 99.9% purity) using a modified Hummers' method (Hummers and Offeman, 1958; Cote et al., 2009a, 2009b). Our previous study proved the characteristics of GO by XRD, UV, Raman and XPS analysis. These results confirmed that GO was successfully synthesized from graphite by the modified Hummers' method and matched well with previous results (Jang et al., 2012, 2013b, Kim et al., 2014). For the synthesis of the various crumpled GR–Au composites, the precursor was prepared with different weight ratios of Au/GR from 1 to 3, while the concentration of the GO was fixed at 0.5 wt% in the colloidal mixture. The crumpled GR–Au composites were prepared from $HAuCl_4 \cdot 3H_2O$ and GO using the ASP method (Jang et al., 2013b). The experimental apparatus for the ASP process consisted of an ultrasonic atomizer, an electrical tubular furnace, and a filter

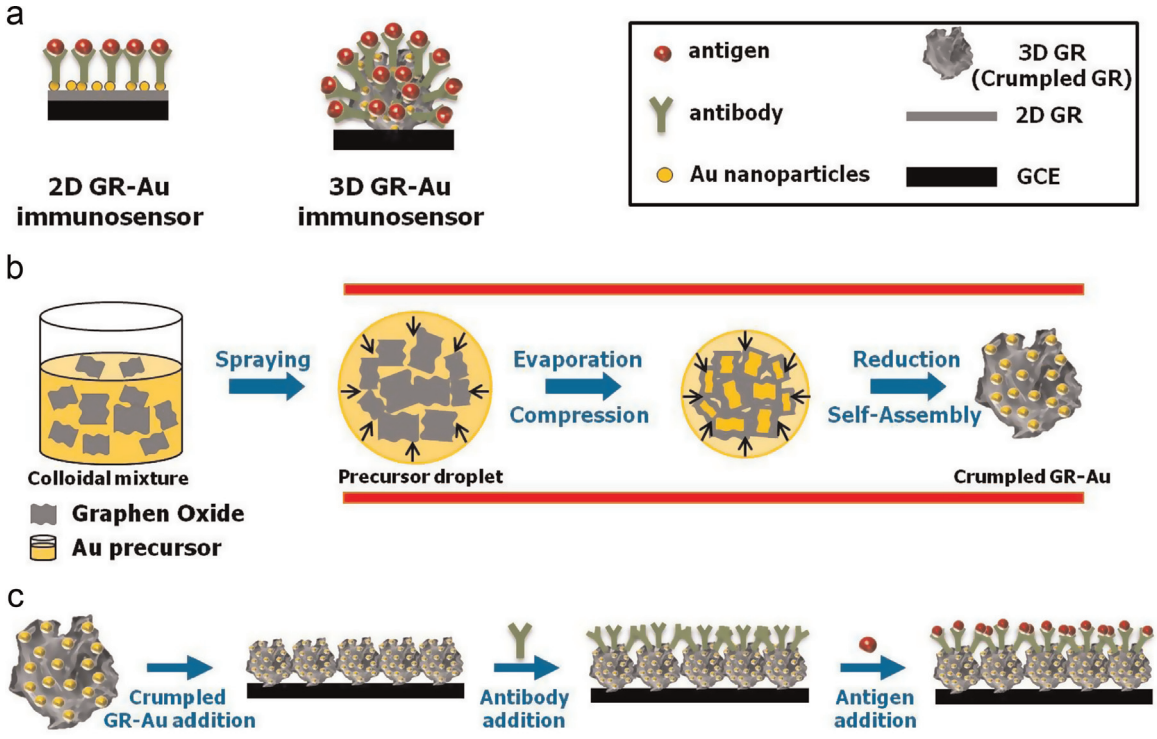

Fig. 1. Schematic illustration of (a) 2D GR–Au and 3D GR–Au electrodes, (b) the formation of crumpled GR–Au composites via aerosol spray pyrolysis and (c) fabricating step of 3D label-free PSA immunosensor using crumpled GR–Au composites.