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A sensitive and selective magnetic graphene composite-modified polycrystalline-silicon nanowire field-effect transistor for bladder cancer diagnosis

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ABSTRACT

In this study, we describe the urinary quantification of apolipoprotein A II protein (APOA2 protein), a biomarker for the diagnosis of bladder cancer, using an n-type polycrystalline silicon nanowire field-effect transistor (poly-SiNW-FET). The modification of poly-SiNW-FET by magnetic graphene with long-chain acid groups (MGLA) synthesized *via* Friedel–Crafts acylation was compared with that obtained using short-chain acid groups (MGSA). Compared with MGSA, the MGLA showed a higher immobilization degree and bioactivity to the anti-APOA2 antibody (Ab) due to its lower steric hindrance. In addition, the magnetic properties enabled rapid separation and purification during Ab immobilization, ultimately preserving its bioactivity. The Ab-MGLA/poly-SiNW-FET exhibited a linear dependence of relative response to the logarithmical concentration in a range between 19.5 pg mL^{-1} and 1.95 μg mL^{-1}, with a limit of detection (LOD) of 6.7 pg mL^{-1}. An additional washing step before measurement aimed at excluding the interfering biocomponents ensured the reliability of the assay. We conclude that our biosensor efficiently distinguishes mean values of urinary APOA2 protein concentrations between patients with bladder cancer (29–344 ng mL^{-1}) and those with hernia (0.425–9.47 ng mL^{-1}).

1. Introduction

Among men and women in the Western world, bladder cancer is the fourth and eighth most common malignancy, respectively. Bladder cancer occurs in the epithelial lining of the bladder (Kirkali et al., 2005). Non-muscle-invasive bladder cancer occurs in approximately 70% of bladder cancer incidents. High-grade stages of muscle-invasive bladder cancer are associated with significant tumor progression and, consequently, increased mortality. Therefore, to determine the optimal treatment, it is critical to accurately detect bladder cancer at an early stage. Traditionally, diagnosis is a complex process based on the experience of a urologist, radiologist or cytopathologist. Diagnostic approaches include cystoscopy, which is invasive and therefore painful, and urine cytology, which is not sufficiently sensitive for the detection of low-grade stages (Araki et al., 2007).

A good biomarker reflects various conditions of a biological system and enables better disease diagnosis and/or treatment outcomes. The biomedical field is highly interested in the development of disease biomarkers, where the biomarker development pipeline generally comprises three stages, which include biomarker discovery, verification, and clinical validation (Surinova et al., 2011). The process usually begins with biomarker discovery based on a limited number of cases and uses deep profiling, which can achieve the identification of thousands of proteins in a biological sample. Prior to the application of potential biomarker candidates in translational medicine, the candidates must be evaluated in 100–1000 samples in the verification and validation phases using high-throughput, sensitive assays. A variety of

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diagnostic tests have been investigated for the detection of bladder cancer. Valid, reliable and inexpensive tests that can be easily and rapidly performed include Raman spectroscopy for diagnosing urothelial carcinoma cell (Shapiro et al., 2011), elastic light scattering for detecting papillary transitional cell carcinoma (Mourant et al., 1995), polymerase chain reaction for telomerase detection (Kim et al., 2013), enzyme-linked immunosorbent assay (ELISA) for MUC1 detection (Ferreira et al., 2008), silicon microring resonators for monitoring fibroblast growth factor receptor 3 and Harvey RAS, nuclear magnetic resonance for measurements of leucine, tyrosine, lactate, glycine and citrate (Cao et al., 2012), electrochemistry for H_2O_2 detection (Hua et al., 2011a; Roberts et al., 2013), chromatography for detecting carnitine C9:1 (Kałuzna-Czaplinska and Jóźwik, 2014), and surface plasmon resonance for Her-2 determination (Tai et al., 2007). Additionally, a variety of candidate bladder cancer biomarkers such as RT112 cell CDH1, FHIT, LAMC2, RASSF1A, TIMP3, SFRP1, SOX9, PMF1 and RUNX3 have been identified but require further validation (Roberts et al., 2013; Shin et al., 2013; Kandimalla et al., 2013). Apolipoprotein A-IIprotein (APOA2) was recently identified as a new biomarker that occurs a highly elevated rates in pooled bladder cancer. The relative difference in the level of APOA2 protein in urine is a highly significant factor (Chen et al., 2010, 2012).

The use of field-effect transistors (FETs) to diagnose bladder cancer from urine specimens has not been reported. Due to the associated advantages of miniaturization and superior sensitivity, significant effort has been expended toward the development of FET biosensors in recent years (Kwon et al., 2012; Lee et al., 2009). High selectivity is achieved by immobilizing antibodies, peptides, and oligonucleotide-based aptamers to bind specific molecules. In this configuration, the binding analyte affects the channel conductivity in a manner that is similar to the effect of voltage application to a metallic gate electrode. However, earlier FETs with planar or two-dimensional surfaces exhibited low sensitivity, limiting their application, particularly for trace analytes. One-dimensional FET channels with nanostructures of nanowires (Li et al., 2013; Chen et al., 2011), graphene (Trung et al., 2014), nanobelts (Oh et al., 2013) or nanotubes (Chen et al., 2014) are extremely attractive due to the depletion or accumulation of carriers in the bulk of the nanoscale structures.

Magnetic and gold nanoparticles are loaded in the channels to increase the surface area, thereby increasing the quantity of immobilized biomolecules (Chen et al., 2014; Allen et al., 2007). Graphene sheets (Gs) are a potential candidate for the modification of SiNW-FETs due to the inherently high surface area and superior conductivity (Sykes and Charles, 2009). However, Gs lack functional groups for the immobilization of biomolecules, thus it is necessary to chemically modify Gs. The best-known functionalization of Gs is Hummers' chemical modification, in which treatment with a strong acid and an oxidant is used to produce various oxygen-containing groups, including short-chain carboxylic acids (Liu et al., 2008). The carboxylic acid group can subsequently form covalent bonds with amine-containing biomolecules *via* the EDC/sulfo-NHS reaction. However, fewer investigations have focused on the effects of chain length on the degree of biomolecule loading and the associated role of steric hindrance. To clarify the effect of the carboxylic acid chain length, Gs with a four-carbon-chain carboxylic acid group were synthesized *via* Friedel–Crafts acylation and compared with the product of Hummers' chemical modification in this study. Additionally, magnetic nanoparticles of Fe_3O_4 were synthesized *in situ* on the carboxylated Gs to enable rapid purification. After immobilizing anti-APOA2 on the magnetic carboxylated Gs and loading on the poly-SiNW-FET channel, a novel bladder cancer biosensor was developed based on a poly-SiNW-FET device. Performing an additional washing process ensured the accuracy of the biosensor measurement by eliminating other charged species. Finally, individual urine samples from age-matched hernia patients and bladder cancer patients were analyzed using the proposed biosensor; the results clearly indicated that the biosensor can distinguish among these two sample types. These results demonstrate that bladder cancer diagnosis is feasible using a modified poly-SiNW-FET device.

2. Experiments

2.1. Patients and materials

Clinical specimens were collected using a previously described protocol (Chen et al., 2010, 2012). Briefly, first-morning urine samples were collected from hernia patient controls and bladder cancer patients into containers that contained a protease inhibitor cocktail tablet (one tablet per 50 mL of urine; Roche, Mannheim, Germany) and sodium azide (1 mM). As controls, first-morning urine samples were collected from hernia patients of comparable age using an identical procedure after admission and before surgical intervention. The collected urine samples were centrifuged at $5000 \times g$ for 30 min at 4 °C within 5 h of collection to remove cells and debris, and the clarified supernatants were stored at −80 °C until further processing. All clinical samples were collected from the Department of Urology, Chang Gung Memorial Hospital, Taoyuan, Taiwan. This study was approved by the Ethics Committee of Chang Gung Memorial Hospital (IRB nos. 102-3232A3 and 99-2188B). All subjects received a description of the study and provided written informed consent. APOA2 and anti-APOA2 were obtained from Abcam, CB, UK. Maleic anhydride (MA) was purchased from TCI. *N*-hydroxysulfo-succinimide sodium salt (sulfo-NHS) was purchased from Fluka. PBS, EDC, goat anti-human IgG (Fc specific)-peroxidase antibody, BSA and MES buffer (pH 6.3) were purchased from Sigma. Acetone, methanol, and 1-methyl-2-pyrrolidone (NMP) were purchased from Tedia. Aluminum chloride and TBO were purchased from Acros, and graphite was purchased from Alfa Aesar. NaOH, $FeCl_2$ and $FeCl_3$ were purchased from Merck.

2.2. Syntheses of GLA and GSA

A 50 mg quantity of graphite as temporarily dispersed in 10 mL of anhydrous NMP with sonication. One gram of MA was dissolved in 40 mL of anhydrous NMP solution under nitrogen, and $AlCl_3$ was then added at a molar ratio of 1:1, 1:3, or 1:6 at 90 °C. After stirring for 3 h, the graphite solution was added to the three MA–$AlCl_3$ solutions and reacted at 160 °C for 48 h. The samples were filtered through 0.1-μm PVDF membranes and washed with methanol and DI water 3 times. The yellow solutions were collected after centrifugation at 3000 rpm. Finally, the products were obtained from the filter cake by filtration through a 0.1-μm PVDF membrane, and the products were labeled GLA11, GLA13, and GLA16. GSA was synthesized from expandable graphite flake using a method modified from Hummer (Liu et al., 2008).

2.3. Syntheses of MGLA and MGSA

A 200-mg quantity of GLA was dispersed in 20 mL of DI water. $FeCl_3$ (4.32 mmol) and $FeCl_2 \cdot 4H_2O$ (6.48 mmol) were dissolved in 380 mL of DI water at room temperature and mixed with the GLA solution under N_2 gas. After heating this solution slowly to 50 °C, and 30 mL of NaOH (0.576 N) was slowly added over 20 min, resulting in a final temperature of 80 °C. The reaction was then rapidly quenched on ice, and 0.1 N HCl was slowly added until a neutral pH was obtained. MGLA was separated from the solution *via* the application of a magnetic field and then was washed with