Analytica Chimica Acta 1000 (2018) 273–282

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Ultrasensitive flexible FET-type aptasensor for CA 125 cancer marker detection based on carboxylated multiwalled carbon nanotubes immobilized onto reduced graphene oxide film

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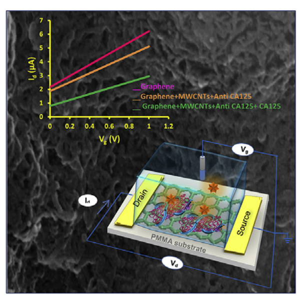
HIGHLIGHTS

- Flexible and novel FET/aptasensor used for detect of ovarian cancer antigen.
- MWCNTs-COOH/rGO integrated with poly-methyl methacrylate as suitable platform for FET.
- With attachment of aptamer on platform an ultrasensitive FET-CA125 aptasensor designed.
- The aptasensor exhibited a wide linear range for CA125 (10^{-9} to 1U/mL) with LOD 0.5nU/mL.
- The FET/aptasensor applied to detect CA125 in human serum with satisfactory result.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 3 October 2017
Received in revised form
5 November 2017
Accepted 7 November 2017
Available online 10 November 2017

Keywords:
Field effect transistor
Graphene
Carboxylated carbon nanotubes
Aptasensor
CA125 cancer marker

ABSTRACT

The development of a novel flexible and ultrasensitive aptasensor based on carboxylated multiwalled carbon nanotubes (MWCNTs)/ reduced graphene oxide-based field effect transistor (FET) has been reported for label-free detection of the ovarian cancer antigen (CA125). The fabricated sensor has a straightforward design based on the noncovalent attachment of MWCNTs/aptamer conjugated onto few layers reduced graphene oxide nanosheets and its integration with poly-methyl methacrylate (PMMA) as a suitable platform for designing flexible field-effect transistors. The surface properties of the aptasensor were characterized using scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM). Under optimal conditions, the proposed aptasensor exhibited a wide linear dynamic range for CA125 (1.0×10^{-9}-1.0 U/mL) with a low detection limit of 5.0×10^{-10} U/mL. The proposed aptasensor was also successfully applied to detect CA125 in real human serum samples. Furthermore, sensor flexibility is also a challenging area in chemical and biological sensors, especially for portable, wearable, or even implantable sensors, so, the reduced graphene oxide-based FET-type aptasensor showed bendable flexibility on the PMMA substrate. In addition, the aptasensor exhibited high sensitivity, selectivity, stability and reproducibility which offers great promise as a high performance and flexible FET-type aptasensor to detect CA125 and other cancer biomarkers in clinical samples and biological fluids.

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https://doi.org/10.1016/j.aca.2017.11.008
0003-2670/

1. Introduction

Ovarian cancers are one of the most lethal gynecological malignancies worldwide and are currently among the most difficult cancers to early diagnose due to the lack of specific signs and symptoms, coupled to the absence of reliable screening strategies. Cancer marker protein CA125, a mucin-like glycoprotein with a molecular weight over 200 kDa, is recommended for clinical use in ovarian cancer screening of high-risk women with ovaries and tracking the disease progression or relapse [1]. The normal level of CA 125 in blood is less than 35 U/mL, and besides ovarian cancer its elevated concentrations has been reported in a number of other malignant conditions such as breast cancer, mesothelioma, non-Hodgkin's lymphoma, etc [2].

Various methods based on electrochemistry [3], surface plasmon resonance [4], quartz crystal microbalance [5], immunoassay [3,6–8], colorimetry [9], fluorescence [10] and mass spectrometry [11] have been developed for the detection of CA 125. Although these methods have gained some degree of popularity and provided some benefits, they suffer from some major drawbacks at the same time, including low sensitivity, long detection time, large sample specimens required and complicated experimental procedures which highly affects the outcome and sensitivity of the analysis [12]. Thus, the identification of new tools able to recognize ultratrace amounts of CA125 with high efficacy and sensitivity is an important challenge in oncology.

Recently, label-free detection of different target analytes (proteins, viruses, oligonucleotides) using a chemiresistive or field effect transistor (FET) sensor has gained tremendous research interest [13]. These sensors do not require fluorescent or electrochemical tags but attain high sensitivity and selectivity [14]. They require a minimal or no sample preparation and make real-time monitoring possible due to faster transduction mechanism. The portability, sensitivity, and overall performance of these devices were improved by using nanostructures such as silicon nanowires, carbon nanotubes and graphene. Graphene not only has excellent electron mobility, thermal conductivity, mechanical strength and large surface-to-volume ratio, but also shows unique tunable ambipolar characteristics and extremely low thermal and electrical noise due to high conductivity and few surface defects. These merits have made graphene an attractive channel material of FET/chemiresistor transducers and also a sensing element for the detection of various analytes [15]. As a result, the 2D graphene-based FET biosensors have been widely used as assay platforms for ultrasensitive and label-free molecular detections. For instance, Kim et al. detected prostate cancer biomarker at femtomolar level through prostate-specific antigen monoclonal antibody-modified reduced graphene oxide (rGO) FET [16]. Recently, a non-enzymatic sensitive H_2O_2 FET biosensor based on polypyrrole nanotubes embedded rGO was fabricated by mixing GO and polypyrrole nanotubes followed by hydrazine reduction [17]. Chang and co-workers developed a rapid method for *Escherichia coli* bacteria detection by thermally reduced graphene oxide FET devices, in which the monolayer GO was produced by ultrasonic-assisted self-assembly [18]. In addition, the GO-based bacterial and DNA sensor utilizing a p-type graphene FET immobilized with bacterial antibody was demonstrated to be ultra-sensitive with single bacterium detection capability [19].

Although most sensing approaches are currently based on antibody-antigen immunoreaction [20–22] biosensing approaches based on aptamers [23–25], synthetic single-stranded RNA or DNA oligonucleotides showing binding affinity to specific targets, have been intensively explored in recent years. Compared with antibodies, aptamers possess some outstanding features, including high specificity of binding affinity, sufficient stability, resistance to harsh environments, smaller size, high purity and very low inter-batch variability that are gained during synthesis and easy modification with electrochemical active markers, optical dyes, enzymes and other desired substances [26]. DNA aptamer-based graphene FET biosensors are highly sensitive, selective electronic devices with distinct advantages over previous aptasensor platforms. Kim et al. recently reported a liquid top-gated graphene FET aptasensor capable of detecting a component of the anthrax toxin protective antigen in the attomolar range. This device performed well with regard to level of detection, dynamic detection range and sensitivity [27]. Recent trends in development of electrochemical aptasensors are focused on application of new transducers able to improve immobilization of aptamers, sensitivity and dynamic range of detection [28]. Multiwalled carbon nanotubes are considered as an important group of nanomaterials and are used for modification of electrode for development of modern electrochemical sensors, owing to their high sensitivity, inherent simplicity, broad potential window, rich surface chemistry, low background current, chemical inertness, miniaturization, and low cost [29]. Recent studies have shown that carbon nanotubes can provide higher surface density of immobilized biomolecules in comparison with traditional planar surfaces and improve significantly the electrochemical reactivity. MWCNTs are appropriate for covalent binding of proteins and mediators. This can be achieved by oxidation of carbon nanotubes which is suitable for chemical attachment of molecules bearing amine groups [30].

Herein, in continuous our previous study [31] we reported a straightforward fabrication methodology of a flexible FET-type aptasensor and demonstrated its capability for sensitive and selective CA125 detection. Simple polishing method was used for deposition of few-layer reduced graphene oxide onto a flexible substrate and integration into the liquid-ion gated FET system via surface engineering. For attachment of anti-CA125 ssDNA onto reduced graphene oxide, aptamer was attached to carboxylated multiwalled carbon nanotubes via N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride and N-hydroxysuccinimide chemistry. To our knowledge, this is the first demonstration of a flexible FET-type aptasensor using reduced graphene oxide to detect CA125 as a cancer biomarker. The graphene-based aptasensor had a strong field-induced response through the binding between antigens and the aptamer, leading to a high sensitivity toward the recognition of a target CA125 biomarker at an unprecedentedly low concentration (5.0×10^{-10} U/mL) and dynamic range up to 1.0 U/mL in 10 mM phosphate buffer solution. The stability and repeatability of the device was investigated and its application for cancer marker detection in human serum of ovarian cancer patients was investigated. Finally, the flexibility of the aptasensor system was investigated.

2. Experimental

2.1. Materials and instruments

Natural graphite powders were supplied from Bay Carbon (SP-1. MI. USA). Carboxylated multiwall carbon nanotubes with purity 95% (30–40 nm diameter), 1 mm length and surface specific area of 480 m^2/g were obtained from Nanolab (Brighton, MA). N-