Analytica Chimica Acta 869 (2015) 68–73

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Single-walled carbon nanotube based transparent immunosensor for detection of a prostate cancer biomarker osteopontin

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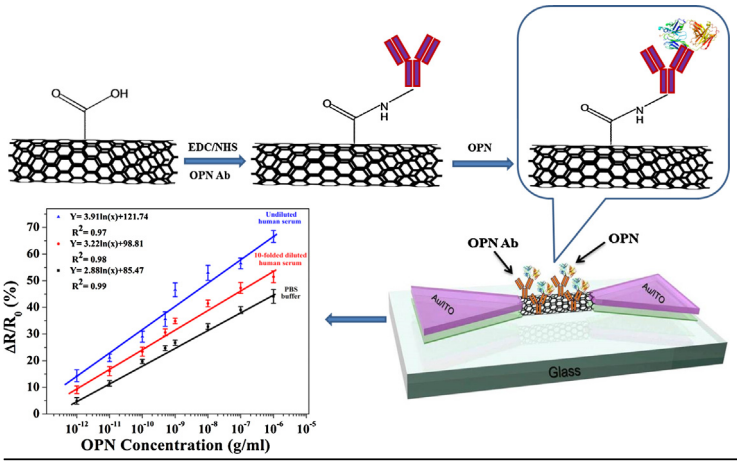
HIGHLIGHTS

- A transparent CNT immunosensor is presented for detection of a prostate cancer biomarker osteopontin.
- This immunosensor showed a highly linear and reproducible behavior from 1 pg mL^{-1} to 1 μg mL^{-1}.
- The limit of detection of the immunosensor was 0.3 pg mL^{-1}.
- This immunosensor demonstrated high selectivity against bovine serum albumin and human serum.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 October 2014
Received in revised form 29 December 2014
Accepted 6 February 2015
Available online 11 February 2015

Keywords:
Cancer
Osteopontin
Carbon nanotubes
Dielectrophoresis
Transparent sensor
Field effect transistor sensors

ABSTRACT

Osteopontin (OPN) is involved in almost all steps of cancer development, and it is being investigated as a potential biomarker for a diagnosis and prognosis of prostate cancer. Here, we report a label-free, highly sensitive and transparent immunosensor based on single-walled carbon nanotubes (SWCNTs) for detection of OPN. A high density of –COOH functionalized SWCNTs was deposited between two gold/indium tin oxide electrodes on a glass substrate by dielectrophoresis. Monoclonal antibodies specific to OPN were covalently immobilized on the SWCNTs. Relative resistance change of the immunosensors was measured as the concentration of OPN in phosphate buffer saline (PBS) and human serum was varied from 1 pg mL^{-1} to 1 μg mL^{-1} for different channel lengths of 2, 5, and 10 μm, showing a highly linear and reproducible behavior (R^2 > 97%). These immunosensors were also specific to OPN against another test protein, bovine serum albumin, PBS and human serum, showing that a limit of detection for OPN was 0.3 pg mL^{-1}. This highly sensitive and transparent immunosensor has a great potential as a simple point-of-care test kit for various protein biomarkers.

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http://dx.doi.org/10.1016/j.aca.2015.02.010
0003-2670/

1. Introduction

Cancer is one of the most life-threatening diseases in humans, and several types of cancers are reported yet among which lung, liver, blood, and prostate cancers are most common and have caused most of the deaths worldwide [1]. Prostate cancer is the second most common cancer in USA after lung cancer [2]. Diagnosis of prostate cancer is a critical limiting factor for this disease because there is no sign of cancer progression for many years in the initial stage. This often results in delayed diagnosis, and hence the cancer can spread from the prostate to surrounding organs without being properly treated. There are several techniques to diagnose prostate cancer including ultrasound, biopsy, and prostate specific antigen (PSA) test. The PSA test is commonly used, and prostate cancer patients generally have abnormally elevated PSA level in serum (>4 ng mL^{-1}) [3]. However, it is well known that PSA tests are prone to both false positives and false negatives [4].

Enzyme-linked immunosorbent assay (ELISA) is a commonly used method for detection of a certain biomarker protein, but this assay has specific requirements such as availability of pure samples, long processing time, special equipment, and trained personnel [5,6]. Therefore, it is highly desirable to make a simple, cost-effective, and highly sensitive immunosensor that can detect a biomarker protein. Recently, carbon nanotube (CNT)- and nanowire-based field effect transistor (FET) biosensors have shown excellent sensitivity and selectivity with a low detection limit of PSA for prostate cancer [7–10]. A CNT-FET based biosensor also showed a detection limit of 30 fM of osteopontin (OPN) for prostate cancer using a genetically engineered single-chain variable fragment antibody [11].

OPN is a potential new biomarker of prostate cancer [12–14]. It is a secreted, 60-kDa phosphoprotein that cancer cells use to facilitate their expansion and can be expressed in a variety of tissues such as bones, brain, kidney, lung, and liver [15]. It is involved in almost all the steps of cancer development, and it is being investigated both as a new therapeutic target and as a potential biomarker for a diagnosis and prognosis of prostate cancer [14–16]. The measurements of free OPN, which is not bound to complement factor *H*, in plasma showed an increase with the stage of prostate cancer [13] although early detection with OPN measurements has not been fully established, requiring more studies [17]. It should also be noticed that OPN measurements are important for the prediction of prostate patient survival and for detection of other cancers. Traditional protein detection methods such as ELISA have shown to be problematic for its quantification [18] and there have been very few studies on the cost-effective, easy to implement immunosensors for OPN detection with comparable or better sensitivity than an ELISA assay.

In this study, we report a label-free, highly sensitive and transparent electrical immunosensor to detect OPN using single-walled carbon nanotubes (SWCNTs). The electrical detection based on CNTs has several advantages such as ease of fabrication, well-understood carbon surface chemistry and simple measurements, which enables miniaturized and inexpensive biosensors. The SWCNTs were deposited between two transparent source and drain electrodes on a glass substrate by dielectrophoresis (DEP), making a channel for OPN attachment and detection. Although chemical vapor deposition (CVD) is a most common technique to grow highly aligned SWCNTs [19] and offers good control on positioning, it requires specialized materials and high temperature for the growth. On the contrary, DEP allows the direct alignment of a high density of SWCNTs at room temperature, and it is generally much simpler and more cost-effective. Monoclonal antibodies specific to OPN were then covalently immobilized on the SWCNT

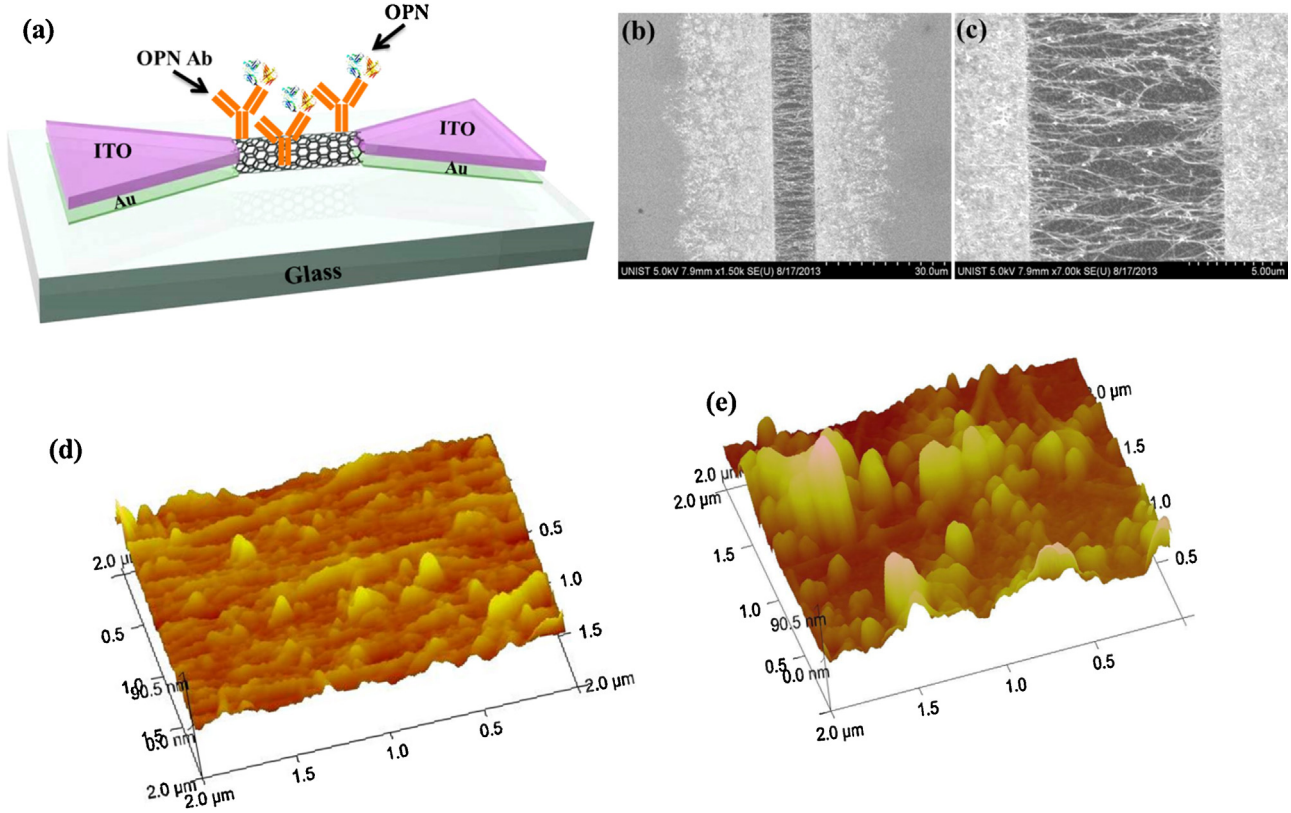

Fig. 1. (a) Schematic of the transparent SWCNT based immunosensors for OPN detection, where OPN antibodies were immobilized on the SWCNT surface between the source and the drain electrodes on a glass substrate, (b) FE-SEM micrographs of SWCNTs between Au/ITO electrodes, (c) FE-SEM micrographs of SWCNTs at higher magnification, (d) AFM images of the SWCNTs deposited between two Au/ITO electrodes, and (e) AFM images of OPN antibody covalently attached to —COOH functionalized SWCNT surfaces.