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A carbon nanotube metal semiconductor field effect transistor-based biosensor for detection of amyloid-beta in human serum

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ABSTRACT

We have developed a carbon nanotube (CNT) film-based biosensor with a metal semiconductor field effect transistor structure (MESFET). A gold top gate was deposited on the middle of the CNT channel and probe antibodies were immobilized on the gold top gate with an antibody-binding protein, protein G or *Escherichia coli* outer membrane (OM) with autodisplayed Z-domains of protein A. These CNT-MESFET biosensors exhibited a higher sensitivity than the CNT-FET biosensor with probe antibodies immobilized using a chemical linker, since the orientation of immobilized antibodies was controlled by the antibody-binding proteins. In addition, nonspecific binding was effectively inhibited by *E. coli* OM. Using the CNT-MESFET biosensors with *E. coli* OM containing Z domain, we detected amyloid-β (Aβ) in human serum, one of the biomarkers for early diagnosis of Alzheimer's disease. Aβ at the level of 1 pg/mL in human serum could be measured in real-time and without labeling, which was lower than a limit of detection for plasma Aβ using an enzyme-linked immune sorbent assay. These results suggested that our CNT-MESFET biosensors might be applicable for an early diagnosis of Alzheimer's disease.

1. Introduction

Immunoaffinity (IA) biosensors based on a carbon nanotube (CNT) with a field effect transistor (FET) structure have been extensively studied for their merits (Allen et al., 2007; Li et al., 2005; Maehashi et al., 2007; Hu et al., 2011), which include label-free detection, real-time monitoring, ultra-sensitivity, and simplicity of apparatus. Target analytes are detected by measuring a change in the electrical conductance of CNT-FET that is caused by binding of target analytes to antigen-binding sites (Fab regions) of antibodies immobilized on the surface of CNTs (Fig. S1). For most CNT-FET IA biosensors, probe antibodies have been immobilized on the CNT surface using a chemical linker, such as pyrenebutyric acid *N*-hydroxylsuccinimide ester (Chen et al., 2001; Oh et al., 2009). However, this linker makes the antibodies bind onto the CNT surface with a random orientation, so that only a small portion of antibodies are immobilized with a proper orientation for binding analytes. Therefore, improved sensor performance may be achieved by increasing the density of antibodies that are immobilized on the sensor surface via the Fc region of antibodies (Fig. S1).

We have developed a semiconducting CNT film-based biosensor with a metal semiconductor field effect transistor structure (CNT-MESFET, Fig. 1(a)). A gold (Au) strip is deposited on the middle of the CNT film channel. Then, a Schottky barrier forms at an interface between the Au strip and the CNT (Oh et al., 2010). For this CNT-MESFET biosensor, probe antibodies can be immobilized on the Au top gate with an antibody-binding protein, such as protein G or protein A. The antibody-binding protein has a high affinity toward the Fc region of antibodies, so the antibodies are immobilized on the Au surface without the involvement of the Fab regions, leading to an increase in the density of probe antibodies with the proper orientation for binding analytes.

Here, we report the fabrication of the CNT-MESFET biosensors using semiconducting CNT films to minimize device-to-device variation (Wang et al., 2009). The fabricated devices exhibited a narrow distribution in the threshold voltage (V_{th}) and the on/off ratio, offering reproducible sensor performance. Probe antibodies were immobilized on the Au top gate of CNT-MESFET using protein G or *E. coli* outer membrane (OM) with autodisplayed Z-domains

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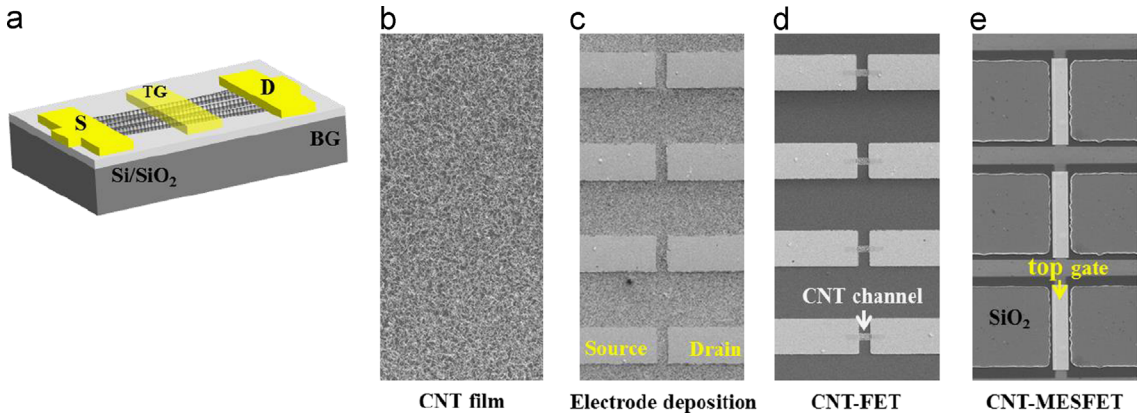

Fig. 1. (a) Schematic diagram of a CNT-MESFET device. (b–e) Fabrication procedure of the CNT-MESFET devices.

of protein A. When target analytes bound to the Au surface with immobilized antibodies, the conductance of the CNT channel changed owing to a potential change of the CNT channel covered with the Au top gate, allowing us to detect target analytes in real-time (Oh et al., 2010). The sensitivity of the CNT-MESFET biosensor was compared with that of CNT-FET biosensors using horseradish peroxidase (HRP) as a model target analyte. Indeed, the CNT-MESFET biosensor with antibody-binding proteins exhibited a higher sensitivity than the CNT-FET biosensor with a chemical linker. In addition, we found that *E. coli* OM with autodisplayed Z-domains of protein A provided a higher sensitivity and a more effective blocking of the unspecific binding in comparison with protein G, which was possibly ascribed to the highly negatively charged *E. coli* OM (Jose et al., 2009, 2010).

Finally, we have applied the CNT-MESFET biosensors modified with *E. coli* OM with autodisplayed Z-domains to the detection of amyloid-β (Aβ) in human serum, a biomarker for early diagnosis of Alzheimer's disease. Although measuring Aβ levels in cerebrospinal fluid (CSF) is now considered to be useful for predicting the severity and progression of the disease, the lumbar puncture to obtain CSF is a more invasive procedure than blood sampling and thus has a critical limitation for repeat testing (Schneider et al., 2009). Additionally, plasma Aβ levels are much lower than CSF Aβ levels (Schneider et al., 2009); therefore ultra-high sensitive biosensors would be required to measure Aβ in human serum and provide reliable test-retest data. Aβ has been usually measured using enzyme-linked immune sorbent assay (ELISA); however, the sensitivity of ELISA is not high enough to detect Aβ in biological fluids at levels below 10 pg/mL (Fagan et al., 2009; Schupf et al., 2008). In addition, several label-free biosensors, such as STM-based (Kang et al., 2009) or reduced grapheme oxide-based biosensor (Kurkina et al., 2012), were reported to measure Aβ in PBS. Although they exhibited ultra-high sensitivity, Aβ in biological fluids was not measured, limiting their practical applications. In contrast, our CNT-MESFET biosensors permitted the real time detection of Aβ at levels as low as 1 pg/mL in human serum. These results suggest that CNT-MESFET biosensors with the protein engineered membrane containing Z domain might be a promising tool for identifying the role of serum Aβ as a biological marker for the early diagnosis of Alzheimer's disease.

2. Materials and methods

2.1. Materials

Polyclonal anti-horseradish peroxidase (HRP, antibody), purified HRP and anti-mouse (goat polyclonal) antibodies conjugated with 20 nm gold nanoparticles were purchased from Abcam (Cambridge, UK). Amyloid-β antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), amyloid-β (1-42, human) (GL Biochem (Shanghai) Ltd., Shanghai, China) and Cys-3 protein G (Bioprogen, Deajeon, Korea) were commercially obtained. Bovine serum albumin (BSA), 3-aminopropyltriethoxysilane (APTES) and 1-pyrenebutanoic acid succinimidyl ester (chemical linker) were bought from Sigma-Aldrich Korea (Seoul, Korea). The 1 × phosphate buffered saline (PBS; pH) was purchased from AMRESCO® and composed of 137 mM NaCl, 2.7 mM KCl, and 10 mM phosphate buffer in 500 mL distilled H_2O. Separated semiconducting enriched single walled carbon nanotubes (SWNTs; 99%) were purchased from NanoIntegris Inc. (Skokie, IL, USA), whose diameter and length were 0.8–1.2 nm and 0.1–1 μm, respectively.

2.2. Fabrication of CNT-FET and CNT-MESFET

A uniform CNT film was prepared on an amine terminated Si/SiO_2 substrate by dropping a solution of separated semiconducting SWNTs in deionized water at 2% weight per volume (w/v) surfactant, sodium dodecyl sulfate (Kim et al., 2011). After leaving for 1 h, the specimen was rinsed with deionized water and dried with N_2 gas. Source (S) and drain (D) (Au (50 nm)/Cr (3 nm)) electrodes were patterned by conventional photolithography and lift-off techniques, followed by rapid thermal annealing at 450 °C for 30 s in a vacuum to form good contacts. Then, CNT channels with a width of 10 μm and a length of 5 μm were defined by an electron beam lithography technique with a negative resist (AR-N-7500, Allresist GmbH, Germany) and then unwanted CNTs were removed using O_2 plasma (Fig. 1(d)). The fabricated CNT-FET devices were characterized by measuring the I_{SD}–V_{SD} and I_{SD}–V_{BG} curves with a semiconductor parameter analyzer (Keithley 4200-SCS). After that, the top gate was fabricated by depositing Au (10 nm) only on the middle of the semiconducting CNT channel to construct CNT-MESFET devices (Fig. 1(e)). Finally, the source/drain electrodes and the CNT parts uncovered by the top gate were passivated by depositing a SiO_2 thin film, leaving only the Au top gate, and then a PDMS well was mounted over the CNT-MESFET.

2.3. Fabrication of E. coli OM with autodisplayed Z-domains of protein A

The autodisplay was performed by transformation using an autodisplay vector constructed by the cloning of the antibody binding Z-domain from *Staphylococcus aureus*, as described in the previous work (Jose et al., 2009, 2010). The OM of *E. coli* with autodisplayed Z-domain was also prepared by using lysozyme reaction and subsequent isolation procedures, as previously reported (Jose et al., 2009, 2010). The OM of intact *E. coli* was also prepared by