Single-walled carbon nanotube field-effect transistor biosensor using small probe molecules

Materials Science
Yuzuru Takamura Lab
1620008
Nguyen Thanh Tung

Background:

Field-effect transistor (FET)-based biosensors offer many advantages such as real-time, label-free, fast response, portability, and high density integration in addition to its high sensitivity. Among FET-based biosensors, single-walled carbon nanotube field-effect transistor (SWCNT-FET)-based biosensor manifests a promising candidate thanking to its quasi-one dimensional SWCNT channel. Every atom is located onto the surface of SWCNT; therefore it is extremely sensitive to small change of surrounding environment. However, FET-based biosensors in general and SWCNT-FET-based biosensor in particular face a critical issue of the Debye shield (physical length of approximately 1 nm induced by counterions in physiological environment) which limits the selection of probe molecules. Besides, conventional probe molecule such as antibody – a product of *in vivo* biological protocol, which is large in size (10 – 12 nm) and suffered from batch to batch activity variation, critical storage condition and high cost, is not suitable for FET-based biosensors. To overcome this problem, the small probe molecule is utilized to bring the binding event to the vicinity of sensing surface of FET in order to enhance the field effect from charged biomolecule target, hence improving the sensitivity of the sensor. In the previous study, small fragment of antibody (such as Fab'2 fragment, Fab fragment) and DNA aptamer were used as small probe molecules integrated into SWCNT-FET biosensor. As a result, the sensitivity of SWCNT-FET sensor could be improved remarkably. In this study, we demonstrated the utilization of small probe molecules such as peptide aptamer and glutamate-binding protein (GBP) integrated with SWCNT-FET. The peptide aptamer is *in vitro* chemically synthesized. Therefore, its activities are uniform regardless of batch with extended storage time; synthesis is quicker and inexpensive; especially it can be flexibly modified to approach wide variety of target while antibody is limited. Compared with DNA aptamer which is made by a combination of 4 nucleic acids, peptide aptamer is a mimic form of a variable loop of antibody, hence peptide aptamer possesses higher specific affinity. The GBP exhibits rapid recognition to transient Glutamate (Glu) – a key neurotransmitter in a central nervous system. Since the current methods for Glu detection are suffered from temporal and spatial resolutions, therefore GBP is a promising candidate to integrate with SWCNT-FET for studying fast process of Glu transmission.

Aim: In this study, we demonstrated the utilization of small probe molecules such as peptide aptamer and GBP integrated with SWCNT-FET (Figure 1). The peptide aptamer was integrated with SWCNT-FET to develop highly selective and sensitive biosensor. The GBP was combined with network SWCNT-FET to develop a sensor for real-time monitoring of transient Glu, aiming for neuroscience application.

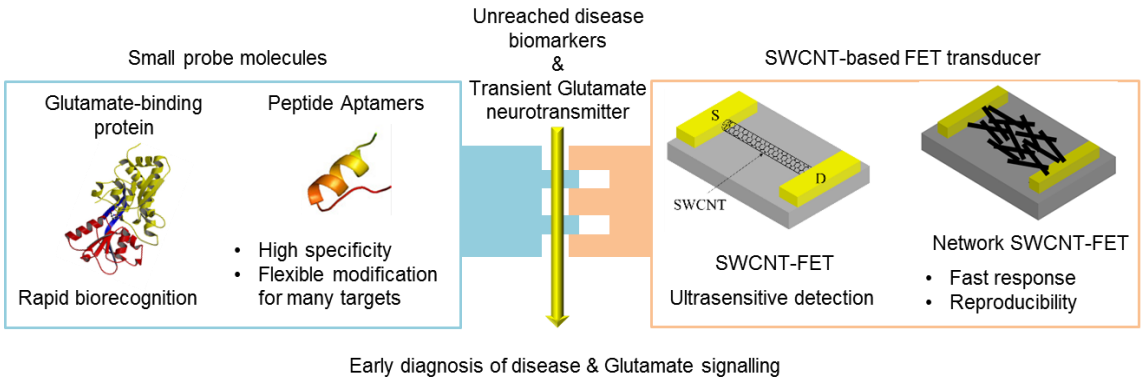

Figure 1: The integration of small probe molecules with SWCNT-based FET.

Experimental:

1. Peptide aptamer-modified CVD-type SWCNT-FET biosensor

First, SWCNT-FETs were fabricated on the Si/SO_2 substrate by growing SWCNTs on Co catalyst using catalytic alcohol chemical vapour deposition (CVD). We call this CVD-type SWCNT-FET. The novel peptide aptamer that specifically recognizes Cathepsin E (CatE) – a useful prognostic biomarker for cancer diagnosis, was utilized as probe molecule. The peptide aptamer was immobilized onto the SWCNT channel via 1-pyrenebutanoic acid succinimidyl ester (PBASE) linker, following by blocking the unreacted PBASE linker with ethanolamine (Figure 2). The fabricated peptide aptamer-modified CVD-type SWCNT-FET sensors were subjected to each CatE concentrations in 1X PBS pH 10 and 10-times diluted human serum, respectively, followed by rinsing thoroughly with 0.0005X PBS pH 4, and then dried with nitrogen gas. Finally, a Parafilm cavity was attached to the device to contain the 0.0005X PBS pH 4 during electrical measurements. To check the selectivity, the fabricated sensors were exposed to the interference molecules such as bovine serum albumin (BSA) and Cathepsin K (CatK).

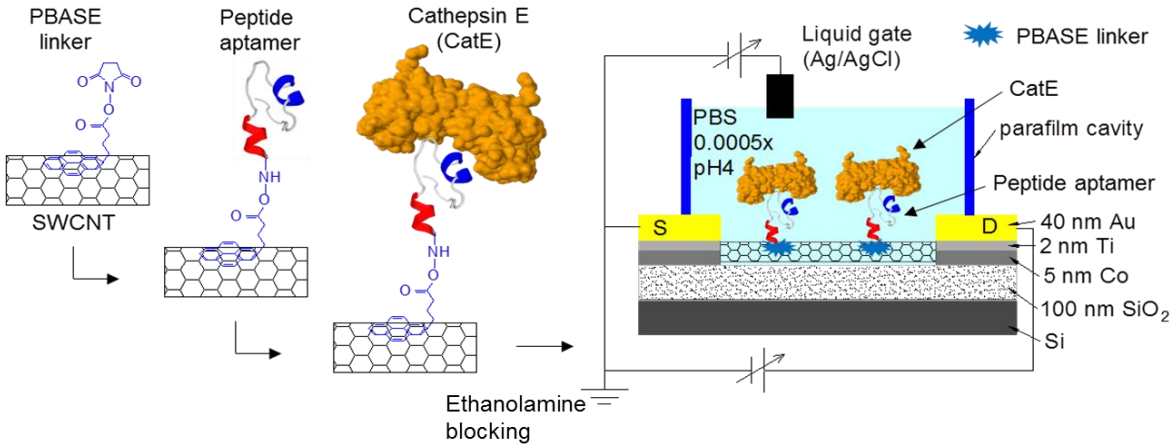

Figure 2: The immobilization of peptide aptamer onto the surface of SWCNT and the operating setup of liquid-gated CVD-type SWCNT-FET device for CatE detection.

2. GBP-modified network SWCNT-FET biosensor

To simplify the process of device fabrication and improve the reproducibility, a semiconducting SWCNT solution was used for the deposition of random network SWCNT channel. This technique is simple and fast, allowing a direct control of network density of SWCNT channel and hence the transistor characteristics. We call this network SWCNT-FET. Then the network SWCNT was functionalized by immobilization of the GBP probe molecule using PBASE linker. Then a Parafilm cavity was attached to the device to contain 0.01X PBS pH 7.5. The fabricated GBP-modified network SWCNT-FET sensor was subjected to various Glu concentrations for real-time measurement. To check the selectivity, the fabricated sensor was exposed to interference molecule such as Dopamine – another neurotransmitter.

Results and discussion:

1. Peptide aptamer-modified CVD-type SWCNT-FET biosensor

Figure 3 (a) shows the response of fabricated peptide aptamer-modified CVD-type SWCNT-FET sensor to CatE in 1X PBS buffer pH 10. The conductance of SWCNT channel decreased with increasing CatE concentrations from 0.1 to 0.6 ng/mL. This decrease in conductance is due to the positive charge of CatE at pH 4 which induces the decrease of hole carrier density inside SWCNT channel by field effect. There was no change in conductance for the CatE concentration above 0.6 ng/mL because all peptide aptamer probe molecules were occupied. There was no significant response of the CVD-type SWCNT-FET device, which was modified with PBASE linker, followed by blocking with ethanolamine, to

1 ng/mL CatE (Figure 3 (b)). This result indicated that the nonspecific binding between CatE and SWCNT was suppressed. Figure 3 (c) shows the comparison between the responses of the fabricated sensors to CatE, CatK and BSA. The result indicates that the fabricated sensors strongly respond to CatE, but negligibly respond to CatK and BSA, exhibiting the specificity of fabricated sensor.

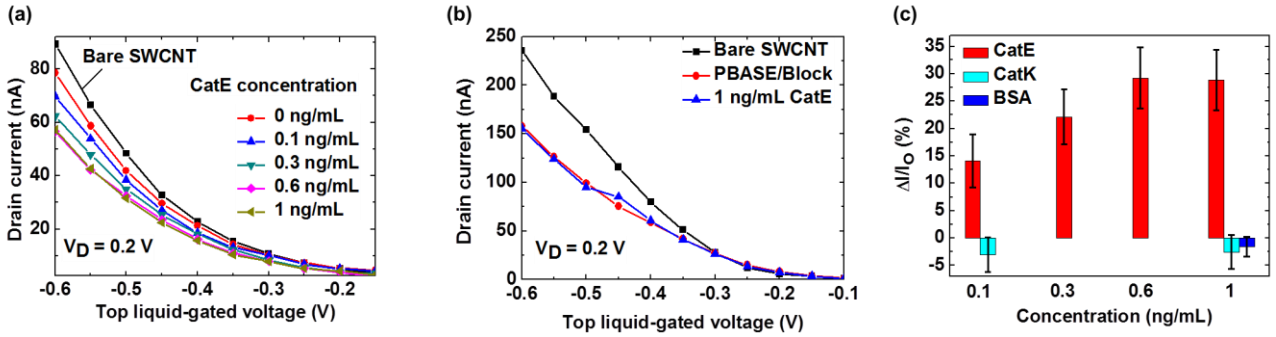

Figure 3: The electrical characteristics of CVD-type SWCNT-FET modified with (a) and without peptide aptamer probe molecule (b) responding to CatE; and (c) the response of peptide aptamer-modified CVD-type SWCNT-FET to CatE, CatK and BSA.

Using the fabricated sensor, we proceed to detect CatE in 10-times diluted human serum. As a result, the sensor responds to CatE from 10 to 100 ng/mL (Figure 4 (a)). In the calibration curve in Figure 4 (b), the intensity of response signals increased with increasing CatE concentration from 10 to 60 ng/mL because of the positive charge of CatE. The lowest detectable CatE of 10 ng/mL in human serum is three time of magnitude lower than that of a conventional ELISA system using a similar peptide aptamer [1]. These results indicated that the use of small peptide aptamer is an effective strategy for realizing highly sensitive and selective FET-based biosensors. Our demonstrated sensor could be a promising platform for near-patient testing and point-of-care testing applications.

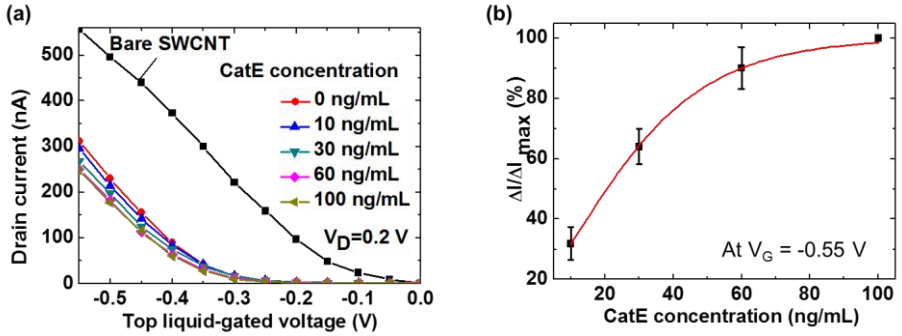

Figure 4: Quantitative detection of CatE in 10-times diluted human serum.

2. GBP-modified network SWCNT-FET biosensor

Figure 5 (a) shows the response of network SWCNT-FET with GBP probe molecule to Glu from 10 to 50 µM (black line) whereas the network SWCNT-FET without GBP shows no significant response to this range of Glu concentrations (red line). This result indicated the nonspecific binding between Glu and network SWCNT was suppressed and the increase in drain current was originated from the binding between GBP and Glu. The increase in drain current is due to the negative charge of Glu and the conformational change of GBP which induces the increase in hole carrier density inside network SWCNT channel. The sharp decrease in drain current at 100 µM is due to the decreased pH by excessive Glu. The fabricated GBP-modified network SWCNT-FET exhibited no significant response to Dopamine (Figure 5 (b)), indicating the high selectivity property of fabricated sensor. These results indicated that our sensor could be a promising candidate for real-time monitoring of Glu at micromolar range which is sufficient for studying Glu signalling.

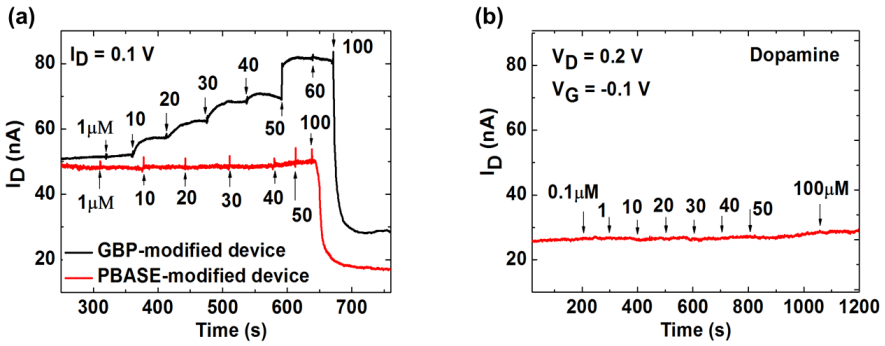

Figure 5: (a) Response of network SWCNT-FET with (black line) and without (red line) GBP to Glu; and (b) Response of GBP-modified network SWCNT-FET to Dopamine.

Conclusion:

We have successfully demonstrated, for the first time, the integration of small probe molecules (peptide aptamer and GBP) into SWCNT-based FETs. The integration of peptide aptamer with CVD-type SWCNT-FET could achive highly sensitive and specific detection of CatE. The integration of GBP with network SWCNT-FET could monitor Glu at micromolar range in real-time. The proposed platforms are promising for clinical application as well as neuroscience study, and be applicable for various target biomarkers.

References:

[1] Kitamura, K. et al. Peptide Aptamer-Based ELISA-Like System for Detection of Cathepsin E in Tissues and Plasma. J. Mol. Biomark. Diagn. 2, 1, https://doi.org/10.4172/2155-9929.1000104 (2011).

Keywords: SWCNT-FET, peptide aptamer, Cathepsin E, Glutamate-binding protein, Glutamate.