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Short communication

A possibility of detection of the non-charge based analytes using ultra-thin body field-effect transistors

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ABSTRACT

Ultra-thin body of p-type field-effect transistors were developed as transducer for biosensors. Changes of conductance resulted from the changes of the surface potentials of ultra-thin body field-effect transistors (UTB-FETs) due to surface chemical modifications were demonstrated. The channel surface of UTB-FETs were modified with *N*-[3-(trimethoxysilyl)propyl]ethylenediamine (AEAPTMS) and then gold nanoparticles (AuNPs) to immobilize the bio-component, the genetically engineered Δ^5-3-ketosteroid isomerase (Art_KSI) or the Art_KSI conjugated with charged reporter (Art_KSI_mA51). The binding of charge-based molecules or nanoparticles has been demonstrated to strongly affect the conductivity of UTB-FETs; the increase or decrease of the conductance depends on the polarity of the immobilized molecules or nanoparticles. A new protocol involving the detection of a non-charged analyte relied on the competitive binding of analyte (19-norandrostendione) and a charged reporter (mA51) with KSI. When exposed to a 19-norandrostendione solution (10 μM), the conductance of Art_KSI_mA51-modified UTB-FET increased by 265 nS (~12%). On the other hand, conductance of Art_KSI-modified UTB-FET showed no distinct change under the same detection conditions.

1. Introduction

Biosensors have found widespread applications in all areas of life sciences research and diagnostics. The main types of signal transducers used in the development of biosensors fall into optical, magnetic, electrical and other categories (Tunnemann et al., 2001; Graham et al., 2004; Pijanowska and Torbicz, 2005). The miniaturization and integration of biosensor platforms in electronic-based methods has become easier than others due to advance in the semiconductor industry.

Numerous research groups have adopted the field-effect transistor as biological-molecular detectors. When appropriate receptors are bound a charged analyte onto the active silicon oxide surface, the accumulation or depletion of carriers (electrons or holes) in the channel of a field-effect transistor can be modulated (Hahm and Lieber, 2004; Patolsky et al., 2004; Kojima et al., 2005; Wang et al., 2005). The main sensing mechanism in electrical-based biosensors relies on a change of the conductance or threshold voltage (V_{th}) which is modulated by the chemical attachment of the molecules to be detected (Hahm and Lieber, 2004; Pijanowska and Torbicz, 2005). Most sensing processes involve charge-based analytes (DNA, protein, antibody and virus) binding or charge products that are formed on the electrical detection platform. However, non-charged analytes cannot be analyzed directly without ELISA-liked assay in the electrical-sensing based system (Kamahori et al., 2007).

During the past decades, steroid hormones assays have been detected based on complex chemicals or enzyme-catalyzed reactions by optical, electrochemical, chromatography or mass detection platform. Steroids are lipophilic, low-molecular mass compounds that are derived from cholesterol, which has various important physiological roles. The major functions of the steroid hormones coordinate the physiological and behavioral responses for specific biological purposes. For instance, the illegal use of 19-nortestosterone as a doping agents in high performance sports is far common. Traces of naturally occurring metabolite have been found in human urine. The concentrations of steroid hormones or their precursors determined by gas chromatography–mass spectroscopy (Wolthers and Kraan, 1999) and liquid chromatography–mass spectrometry (Ma and Kim, 1997) have been developed and well documented. Nowadays, the new technologies, including enzyme-linked immunosorbent assay (Hungerford et al., 2005), fluorescence resonance energy transfer (De et al., 2005) and surface plasmon resonance (Kanayasu-Toyoda et al., 2005) are successfully employed to analyze steroids quantitatively.

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For the electrical-based biosensor, the sensing protocol all involves the introduction of charge analytes. In this investigation, the artificial enzyme (Art_KSI) pre-conjugated with a charged reporter (mA51) was immobilized over the surface of ultra-thin body field-effect transistor (UTB-FET). The hydrophobic interaction promotes the binding of the charged reporter onto the steroid-binding site. Consequently, the charged reporter operates at the equilibrium of the population of this ligand inside or outside the binding site. This charged reporter, when exposed to the polar environment, would carry more negative charges due to abstraction of proton by buffer. As 19-norandrostendione (19-NA) is present in the system, the charged reporter is competitively expelled from the steroid-binding site and presumably exposes to the surface of UTB-FET. Consequently, a clear change of conductance in the UTB-FET can be observed.

2. Experiments

2.1. Sensor design

To construct the bio-component of this sensor, KSI, containing 125 amino acid residues, from *Pseudomonas testosteroni* (Klawahara et al., 1962; Kuliopulos et al., 1987) was genetically reconstructed with eight mutational points (Y55F, K60R, F86C, F88G, K92R, K108R, K119R and A125K) by polymerase chain reaction. The sole cysteine (Cys-86) and lysine (Lys-125) in the artificial KSI are designed for the conjugation of the charged reporter on protein and for the protein immobilization on UTB-FET, respectively. This new biosensing mechanism of a non-charged analyte (Fig. 1) is based on the competitive binding of analyte, 19-NA, and the charged reporter onto the steroid-binding site in KSI. The charged reporter (as shown in Fig. S1) tethered in artificial KSI was used to describe the enzyme-ligand binding interaction on the UTB-FET. The conjugation of the charged reporter and protein was analyzed by mass spectrometry, as shown in Fig. S2.

2.2. Fabrication of UTB-FETs

In order to improve the change of conductance of UTB-FETs, 50-nm top silicon layer of silicon-on-insulator (SOI) wafer (BOX = 150 nm) was reduced to 10 nm by cyclic thermal oxidation. The patterns at active layer are defined by optical lithography and followed by BOE process of the mask oxide layer. Top silicon layer is then etched down to the buried oxide with anisotropic wet etching in 25 wt.% tetramethylammonium hydroxide (TMAH) solution at 50 °C. Second optical lithography was performed to align the active layer on the sample. A metal (Ti/Au) deposition was followed with a lift-off process in acetone. The contacts of samples were activated by thermal annealing at nitrogen atmosphere at 350 °C for 5 min.

2.3. Immobilization of the bio-component on UTB-FET

In biosensing applications, the preparation of the surface of UTB-FET channel is very important for enzyme immobilization. UV–ozone plasma was adopted to activate the channel surface of the UTB-FET sample. The density of active hydroxyl groups were enhanced by UV–ozone plasma treatment for 10 min. Then, the UTB-FETs were immersed into the *N*-[3-(trimethoxysilyl)propyl]ethylenediamine (AEAPTMS) solution to produce amino groups on the channel surface. The AEAPTMS-modified UTB-FETs were further incubated with the AuNPs (Sheu et al., 2005) solution for 24 h to anchor AuNPs through the ionic interaction of carboxylate moieties and the amino groups on AuNPs and UTB-FETs, respectively. The resulting UTB-FETs were then immediately treated with *N*,*N*′-dicyclohexylcarbodiimide (DCC) solution for 4 h at room temperature to activate the accessible carboxyl groups for further protein immobilization. Following the DCC activation, the device was extensively washed with dichloromethane to remove excess DCC. To perform protein immobilization, 20 μl of Art_KSI_mA51 or Art_KSI (1 mg/ml) was dropped onto the channel surface of UTB-FETs. After overnight incubation at 4 °C, the device was extensively washed with Tris–HCl buffer (50 mM, pH 7.5) to remove the excess protein. The device was then kept at 4 °C until use. For steroid measurement, 10 μl of 19-NA solution (10 μg/ml) was dropped onto the surface of UTB-FETs.

3. Results and discussion

The hydrophilic surfaces of channel were initially introduced with hydroxyl groups by UV–ozone plasma treatment, and the

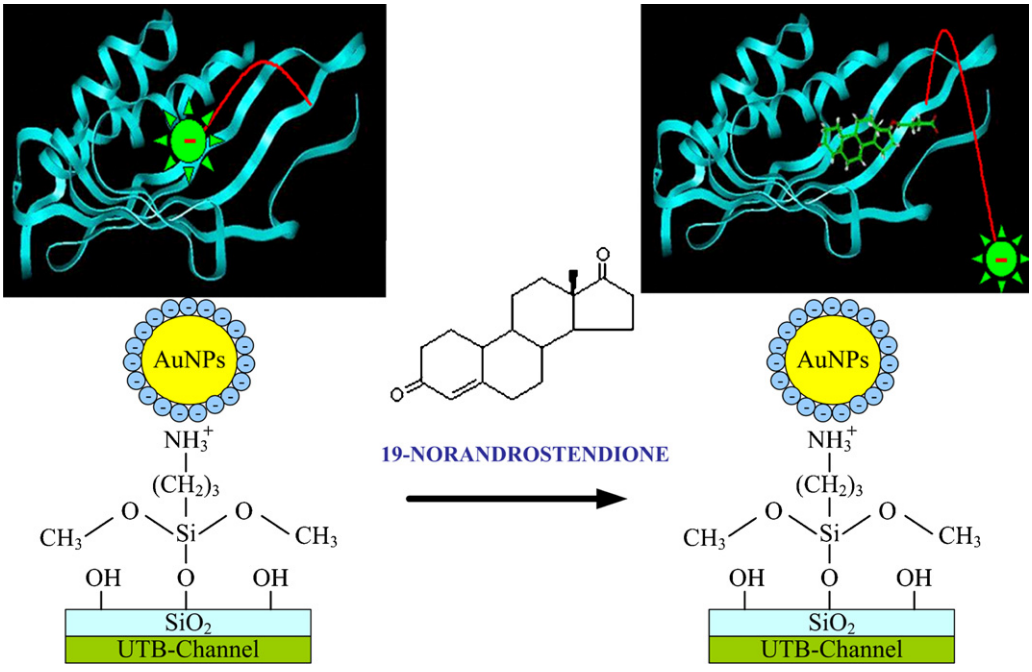

Fig. 1. The scheme of proposed detection mechanism of 19-norandrostendion using UTB-FETs.

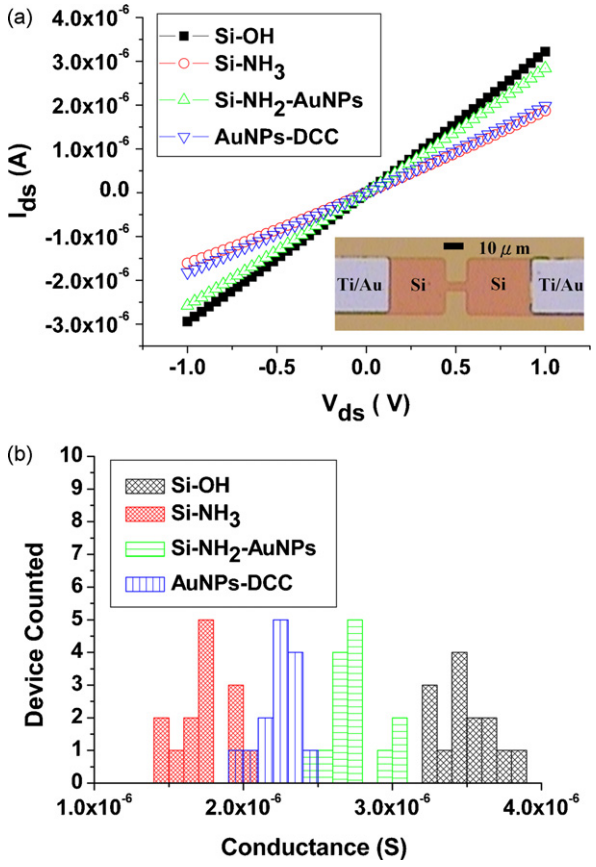

Fig. 2. Electrical properties of UTB-FETs after surface modifications. (a) I_{ds}–V_{ds} measurements on the same p-type UTB-FET after various surface modifications; *insert*: the optical top-view image of a p-type UTB-FET. Dimensions of device channel, width = 5 μm, length = 10 μm and thickness = 10 nm. (b) Histogram of conductance of p-type UTB-FETs after surface modifications.

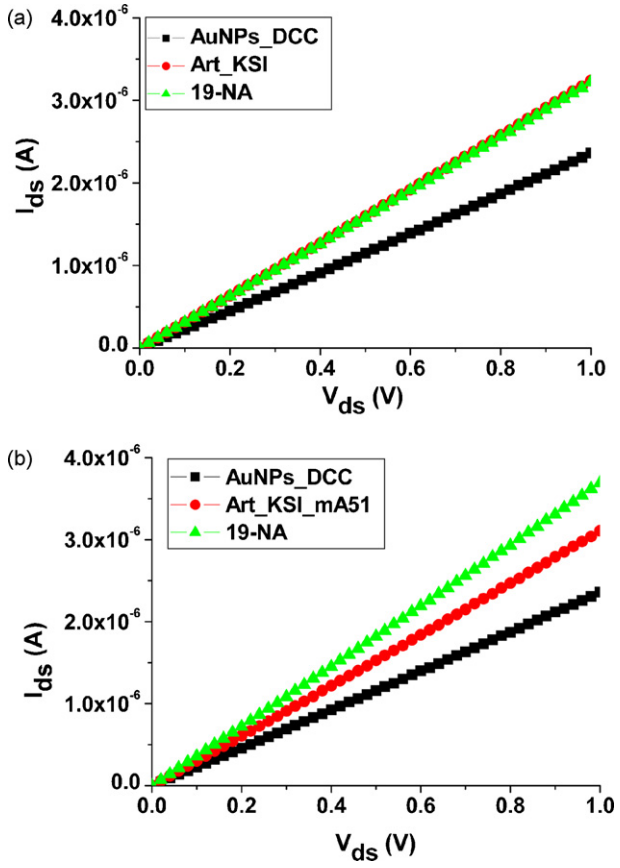

Fig. 3. I_{ds}–V_{ds} curves of a typical p-type UTB-FET. (a) The electronic response of Art_KSI-modified p-type UTB-FET on detection of 19-NA. The conductance showed no distinct change after supply of 19-NA. (b) The electronic response of Art_KSI_mA51-modified p-type UTB-FET on detection of 19-NA. A significant change in conductance was observed after supply of 19-NA.

excess organic reagents were removed by UV–ozone plasma cleaning. The organic monolayer of AEAPTMS was covalently linked to the surface of UTB-FET via Si–O–Si bonds. The AEAPTMS-modified UTB-FETs were then subjected to anchoring gold nanoparticles (AuNPs) (~15 nm in diameter) through electrostatic interaction. The carboxyl groups surrounded AuNPs were activated by dicyclohexylcarbodiimide to produce the key intermediate, the *O*-acylisourea, which can be considered as a carboxylic ester with an activated leaving group. After DCC activation, Art_KSI was covalently linked to the surface of AuNPs through the formation of amide bond with the N-terminal amino group of protein or the amino side-chain of C-terminal lysine. Conductance measurements were independently performed on a device with 14 UTB-FETs after the sequential modifications with AEAPTMS, AuNPs, DCC and protein. Fig. 2(a) demonstrates the current–voltage (I_{ds}–V_{ds}) curves of a UTB-FET with various steps of modifications at a fixed bottom gate bias of $V_g = -10$ V. The inset displays the optical image of the device structure of a UTB-FET. The channel length, channel width and channel thickness are 10 μm, 5 μm and 10 nm, respectively.

The conductance of UTB-FETs responded to the charge state on the channel surface. AEAPTMS modified the silicon dioxide layer to generate an amine-derivatized surface which converted the surface potential of the UTB-FET from negative to positive state and affected the conductance of UTB-FETs from 3.09 ± 0.14 μS to 1.74 ± 0.09 μS, as plotted in Fig. 2(a).

The binding of negatively charged AuNPs onto the amine-derivatized surface acts as a negative gate voltage, which increases the conductance by the accumulation of holes in the channel. These results showed that the attachment of about 6.5×10^4 AuNPs (estimated by atomic force microscope) substantially enhances the conductance of a UTB-FET, as shown in Fig. 2(a). The increases in the conductance, from 1.74 ± 0.09 μS to 2.72 ± 0.19 μS, upon AuNPs attachment is simply an electrostatic gating effect on UTB-FET.

Fig. 2(a) also shows the electronic characteristic of the DCC on device. DCC, labeled on AuNPs via the formation of covalent bond with the carboxyl group, depleted the electronic holes in the UTB-FET channel resulting in a decrease of conductance from 2.72 ± 0.19 μS to 1.90 ± 0.13 μS. Clearly, the chemical modification on channel surface dramatically affected the conductance of UTB-FET devices. To examine the reproducibility of these observations, we have performed measurements over 14 UTB-FETs in a device. The histogram of conductance measurements was shown in Fig. 2(b). All devices behaved with same tendency in the changes of conductance after modifications.

Successful immobilization of protein on the DCC-activated device can be easily confirmed from the observation of conductance change. As shown in Fig. 3, both the Art_KSI-modified and the Art_KSI_mA51-modified UTB-FETs enhanced their conductance as compared to that of DCC-activated one. Art_KSI-modified UTB-FET, served as control experiments, showed no considerable change in conductance with or without 19-NA, as shown in Fig. 3(a). However, the exposure of an Art_KSI_mA51-modified UTB-FET to a 19-NA solution (10 μM) increased the conductance by 265 nS (~12%), as plotted in Fig. 3(b). The increase in the exposure of mA51 in buffer produced an effective negative gating effect on p-type UTB-FET due to competitive binding of mA51 and 19-NA with KSI. This new

sensing model can be adopted to analyze the non-charged analytes with UTB-FET devices. All conductance measurements were performed on a device containing 14 UTB-FETs. The sensitivity or the relative change in conductance ($1 - G/G_0$) derived from the control and sensing devices in the presence of 10 μM 19-NA were calculated and shown in Fig. S3. The value for Art_KSI_mA51-modified UTB-FET (-0.118 ± 0.035) was much more significant than that for Art_KSI-modified UTB-FET (0.019 ± 0.027).

4. Conclusions

The silicon dioxide surface of the p-type UTB-FET was sequentially modified with AEAPTMS, AuNPs, DCC and Art_KSI_mA51 for biosensing. This novel nano-bio-device was designed for the detection of a non-charged steroid. The sensing mechanism is based on the competitive binding of the analyte and mA51 (the charged reporter) with the artificial KSI. We have successfully demonstrated the feasibility of this design. Without extensive effort on optimizing the device, 10 μM 19-NA can be unequivocally detected. Further study will be carried out for improving the sensitivity of detection.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2008.02.024.

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