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Acetylcholine biosensors based on layer-by-layer self-assembled polymer/nanoparticle ion-sensitive field-effect transistors

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Abstract

In this paper, the fabrication and characterization of acetylcholine biosensors based on nano self-assembled ion-sensitive field-effect transistors (ISFETs) are demonstrated. The fabrication is implemented with a very low-cost layer-by-layer nano self-assembly technique. The self-assembled polyaniline thin films work as the semiconducting channel material, while silicon dioxide (SiO_2) nanoparticle thin films serve as the gate dielectric material. Both the silicon wafer and flexible plastic transparency have been applied as the substrate materials. A polyaniline ISFET on silicon wafer operates at a low-voltage range and has a mobility of 1.49 cm^2/V s. Acetylcholine in a concentration as low as 1 μM could be detected with this sensor. A polyaniline ISFET on plastic substrate could detect acetylcholine concentration down to 10 μM. The results presented in this paper suggest a route to fabricate inexpensive and flexible ion-sensitive field-effect transistors for biosensing applications.

Keywords: Nano self-assembly; Acetylcholine; Biosensor; Field-effect transistor

1. Introduction

Acetylcholine (ACh) is a chemical transmitter in both the peripheral nervous system and central nervous system of many organisms including humans. Acetylcholine neurotransmission is considered to play a critical role in human during the processes such as behavioral activity, arousal, attention, learning, memory, etc. An abnormally short supply of acetylcholine is associated with Alzheimer's disease, which ranks the fourth in the causes of death among adults. Therefore, the sensing of acetylcholine concentration is important and of great interest. Some methods can be used to detect the concentration of acetylcholine, for example, electrospray ionization mass spectrometry (EIMS) [1] and silicon-based biosensors [2]. However, both the EIMS and the silicon-based biosensors are relatively expensive because the EIMS is a bulky instrument and the fabrication of silicon-based biosensors is complicated and costly. Polymers and nanoparticles are alternatives to silicon for making low-cost electronic devices and systems. In this report, polymer- and nanoparticle-based field-effect transistors are fabricated and characterized for acetylcholine biosensing.

Biosensors are analytical devices incorporating a biological material with a physical transducer or transducing microsystem. The transduction mechanism may be optical, electrical, thermal, piezoelectric, magnetic, etc. One of the most popular biosensors is based on the ion-sensitive field-effect transistor (ISFET), which was first introduced by Bergveld [3]. The advantages of ISFET-based biosensors include high sensitivity, real-time, and label-free detection of a wide range of chemical and biological species. Since ISFET biosensors can be produced by the integrated circuit production method, they could be reduced in size and mass-produced. They are not only known as one of the most important miniaturized biosensors, but also as devices to bridge the gap between microelectronics and biotechnology.

Recently, layer-by-layer (LBL) nano self-assembly technique has attracted much attention since the introduction by Decher [4]. The LBL process involves alternating immersion of a substrate into aqueous solutions of polycations and polyanions. With each immersion, a polyion layer is deposited and the surface ionization of the substrate is reversed, allowing a subsequent layer with opposite charge to be deposited. Multi-layer thin films made of interesting charged materials such as nanoparticles, conjugated polymers, DNA, proteins, etc. can be formed

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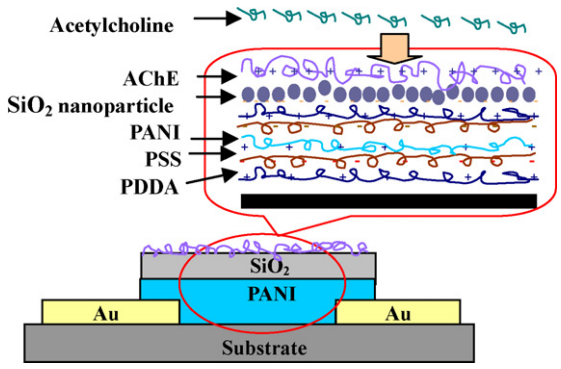


Fig. 1. The schematic diagram of the ACh-sensitive ISFET.

with controlled thickness in nanometer scale. Using LBL self-assembly, it is very simple and inexpensive to produce organized films similar to the ones obtained with the sophisticated and expensive molecular beam epitaxy technology. The versatility, relative ease of preparation, ultra low cost, and potential for scale-up have made LBL self-assembly a viable chemical approach for the fabrication of nanostructure devices. There are several self-assembly techniques, such as Langmuir–Blodgett (LB) films, self-assembly of monolayers (SAM), and LBL self-assembly. Compared to LB technique and SAM technique, the LBL films exhibit a much larger thermal and mechanical stability, and can be prepared up to hundreds of layers. LBL self-assembly is proving to be a useful and versatile technique for the formation of multi-layered thin films with a wide range of electrical, optical, and biological properties. It can find applications including optical diode [5], biosensor [6], etc.

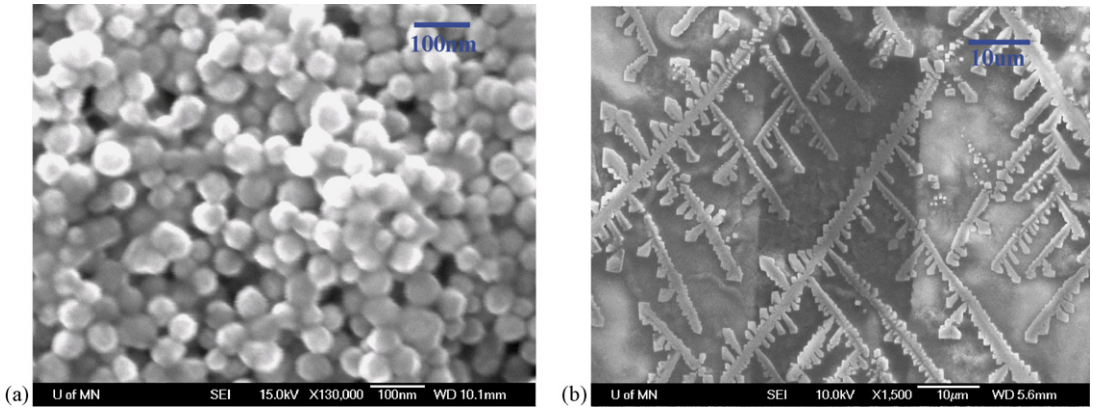
Recently, we have reported the fabrication of LBL self-assembled polymer/nanoparticle ISFET on silicon substrate for acetylcholine biosensing [7]. In this paper, the LBL self-assembled polymer/nanoparticle acetylcholine-sensitive ISFET have been fabricated on both the silicon and flexible plastic substrate. Conjugated polymer, polyaniline (PANI), has been self-assembled as the semiconductor channel material. Fig. 1 shows the schematic diagram of the acetylcholine-sensitive ISFET. The structure of our ISFET is similar to a typical thin-film transistor except that the gate electrode is replaced with a silver/silver chloride (Ag/AgCl) reference electrode. The channel width of the ISFET is 1000 μm, and the channel length of the ISFET is 25 μm. Gold (Au) serves as the source/drain electrodes. LBL self-assembled PANI multi-layers works as the channel, and the self-assembled SiO_2 multi-layers acts as the gate dielectrics. The top self-assembled thin films are acetylcholine esterase that behaves as the bio-receptor for the biosensor. The sensing principle of the acetylcholine ISFET biosensor is based on the biocatalyzed hydrolysis of acetylcholine in the presence of acetylcholine esterase (AChE), as shown in Eq. (1). The hydrolysis results in acetic acid that changes the PH of the solution, and can be detected by the ISFET:

$$(CH_3)_3N^+CH_2CH_2OCOCH_3 + H_2O \xrightarrow{AChE} (CH_3)_3N^+CH_2CH_2OH + HOCOCH_3 \quad (1)$$

2. Experimental

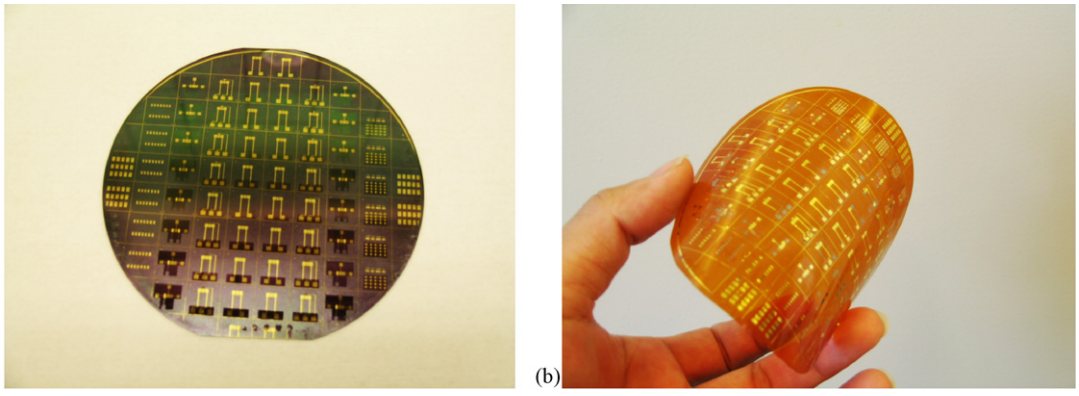
The polyions involved in the fabrication process were positively charged poly(dimethyldiallyl-ammonium chloride) (PDDA) and negatively charged poly(styrenesulfonate) (PSS). The concentration of PDDA and PSS aqueous solutions were 15 mg/mL. Both solutions were doped with 0.5 M NaCl to increase their ionized strength. Polyaniline with sulfuric acid (2.5 mg/mL) was used as the channel material. SiO_2 nanoparticles 40–60 nm in diameter were used as the gate dielectric material. The concentration of SiO_2 nanoparticle solution was 5 mg/mL. The acetylcholine esterase from electric eel and acetylcholine chloride were prepared from de-ionized (DI) water. All the above materials were purchased from Sigma–Aldrich Corporation, except that the SiO_2 nanoparticle dispersion was from Nissan Chemical Corporation.

The nano self-assembled ISFETs were built on both a standard 4 in. silicon wafer with a layer of SiO_2 300 nm thick, and a flexible plastic substrate. First, chrome (100 nm) and gold (200 nm) were deposited on the substrate with e-beam evaporation, and then patterned by optical lithography to form the source/drain electrodes. Next, photoresist was spin-coated on the substrates and patterned to open the area for the test contact electrodes. Prior to the layer-by-layer self-assembly, the substrates were put into the O_2 plasma for 30 s to clean the photoresist residue inside the opening window. The substrates were then alternately immersed in aqueous PDDA and PSS solutions, in a sequence of [PDDA (10 min) + PSS (10 min)]$_3$. These three bi-layers of PDDA/PSS served as the precursor layers that helped to enhance the subsequent adsorption of conjugated polymers and nanoparticles. PDDA and PSS also worked as the sandwich layers between two neighboring nanoparticle, polymer, or AChE layers, depending on the charge polarization of the solution. Between two immersions of the substrates into solutions, there was intermediate rinsing using DI water for 1 min to remove the residue on the previous layer from the surface. Following the precursor layers, five layers of PANI polymer thin films were coated on the entire substrate surface in the sequence of [PANI (10 min) + PSS (10 min)]$_5$. Next, the SiO_2 nanoparticle dispersion were coated in the sequence of [PDDA (10 min) + SiO_2 (4 min)]$_6$ to produce a gate dielectric thin film. The growth step of the SiO_2/PDDA bilayer was 30 nm. Before immobilizing AChE on the top surface of SiO_2 nanoparticle thin film, the substrates were soaked in an acetone solution with ultrasonic agitation to lift off the self-assembled materials not in the target window. Finally, AChE was immobilized on the top of the SiO_2 film in the sequence of {PDDA (10 min) + [PSS (10 min) + AChE (10 min)]$_3$}. Scanning electron microscopy (SEM) was used to investigate the self-assembled nanoparticle and AChE thin films. Fig. 2a and b shows images of self-assembled SiO_2 nanoparticle thin films and AChE enzyme on the top of channel region. Fig. 3a and b shows the photos of fabricated transistors on silicon substrate and flexible substrate, respectively.



(a) (b)

Fig. 2. (a) The SEM image of SiO_2 nanoparticle thin film. (b) The SEM image of AChE enzyme immobilized on the top of channel region.

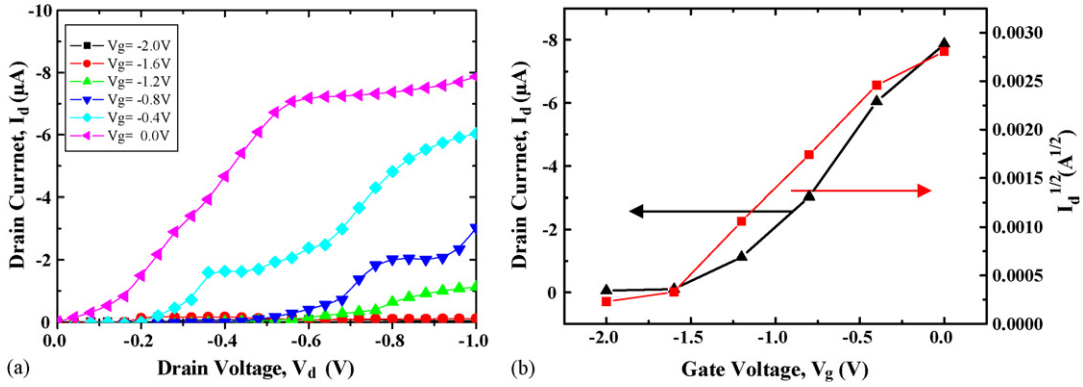


(a) (b)

Fig. 3. (a) The photo of ISFETs on silicon substrate. (b) The photo of ISFETs on flexible plastic substrate.

3. Results and discussion

A HP 4156B semiconductor parameter analyzer is used to characterize the electrical behaviors of the fabricated ISFETs. Ag/AgCl is used as the reference gate electrode. Fig. 4a shows the output characteristic of a PANI ISFET on silicon substrate at an acetylcholine solution with a concentration of 10 mM. This ISFET behaves like a traditional metal oxide semiconductor field-effect transistor (MOSFET). At higher positive gate voltage, the drain current is higher, which indicates that the LBL self-assembled PANI ISFET is functional as an n-type transistor. It is at the "normal-on" state at zero gate voltage, and then works at depletion mode with a negative bias. Electron polarons and bipolarons are the main charge carriers in the n-type PANI semiconductor thin films. This is different from the spin-coated PANI transistor that work as a p-type transistor [8,9]. These two different charge carrier mechanisms are due to the difference between the layer-by-layer self-assembled PANI thin film and the spin-coated PANI thin film. In the spin-coated PANI thin film, only the PANI solution was deposited on the channel region between the source and the drain electrodes. However, in the layer-by-layer self-assembled PANI, the PANI



(a) (b)

Fig. 4. (a) The output characteristic and (b) transfer characteristic of the PANI ISFET on silicon substrate in an acetylcholine solution with a concentration of 10 mM.

was sandwiched into the PSS polyelectrolyte by electrostatic force. The network of mixed PANI and PSS results in strong electron–hole asymmetry in PANI thin film. This n-type asymmetry with respect to polaronic effects, which has been predicted by Libert et al. [10], can contribute to the n-type field-effect characteristics. The n-type field-effect in LBL self-assembled PANI thin films has also been observed by Paloheimo et al. [11].

Fig. 4b shows the transfer characteristic of the same PANI ISFET in the acetylcholine solution (10 mM). The extracted threshold voltage is −1.8 V. The calculated mobility based on the traditional MOSFET theory is 1.49 cm^2/V s. There are 40 ISFETs fabricated on one substrate. The characterized mobility of these devices is within the range from 1 to 5 cm^2/V s. It is noticed that the general polyaniline mobility is within the range from 10^{-5} to 10^{-4} cm^2/V s [11]. Our PANI-based ISFET has a higher mobility, and were investigated carefully.

It is observed that the gate leakage current is higher when the gate voltage is zero, but reduces rapidly when the gate voltage negatively increases. This implies that there are hydrogen ions passing through the gate dielectric layer and participating in the charge transport at zero or low negative gate bias. Since the diameter of the hydrogen ion is less than 0.3 nm, it can easily penetrate the small spaces among the SiO_2 nanoparticles. Since polyions (PDDA and PSS) within the multi-layer films are also conductive in solution, the total charge carriers in the channel should include hydrogen ions, electron polarons from the gate-affected PANI semiconductor thin films and the polyions between the PANI thin films. These carriers greatly increase the channel's conductivity as well as the charge transport ability. Therefore, the calculated mobility should be an "equivalent mobility" that takes into account all the charge carriers. The equivalent mobility could be much higher than a similar transistor in atmosphere ambient. Similar behavior is also observed by Sandberg et al. in an all-polymer field-effect transistor, which has a high mobility (>100 cm^2/V s) in a humid environment, about four to five orders higher than a typical polymer transistor using poly(3-hexylthiophene) as channel material [12].

Fig. 5a shows the sensitivity of a PANI ISFET for different concentrations of acetylcholine when the gate voltage is 0 V and the drain voltage is −1 V. At lower concentration of acetylcholine, the drain current is also smaller. This is because lower concentration of hydrogen ions can be generated during the reaction between acetylcholine and acetylcholine esterase when the concentration of acetylcholine is lower. This has also demonstrated how the device can be effectively used as a biosensor. The ISFET could detect a concentration change of acetylcholine down to 1 μM.

Fig. 5b shows the response time of the PANI ISFET with respect to the open circuit potential change for different concentrations of acetylcholine. The voltage between drain electrode and the Ag/AgCl reference electrode escalated with an increased acetylcholine concentration. When the concentration of acetylcholine was changed from 10 to 100 nM, the change of gate potential was negligible. However, when the concentration of acetylcholine was 1 μM, the increase of the gate potential was obvious. Further increases in the concentration of acetylcholine also greatly increased the gate potential. Since a change of the gate voltage will affect the channel conductivity, it is obvious that the ISFET could detect variations of acetylcholine concentration larger than 1 μM.

The PANI ISEFT on flexible substrate is also applied for acetylcholine biosensing. Fig. 6a shows the output characteristic of the PANI ISFET in the acetylcholine solution with a concentration of 10 mM. It shows that the PANI ISFET on plastic substrate functional similar to the one on silicon substrate, but with less ideal characteristic. The drain current cannot saturate well at higher drain voltage. Fig. 6b shows the drain current versus the acetylcholine concentration when the drain voltage is fixed at −1 V and the gate voltage is fixed at 0 V. The drain current is higher when the acetylcholine concentration is higher. This shows that the PANI ISFET on plastic substrate can be used to detect the acetylcholine concentration. However, when the acetylcholine concentration is less than 1 μM, the drain current tends to fluctuate and no longer decreases stably. This shows that the PANI ISFET on plastic substrate has resolution about one order smaller that the PANI ISFET on silicon substrate. Compared the PANI acetylcholine-sensitive ISFET on silicon substrate with the one on plastic substrate, the ISFET on silicon substrate has better performance in terms of output characteristic

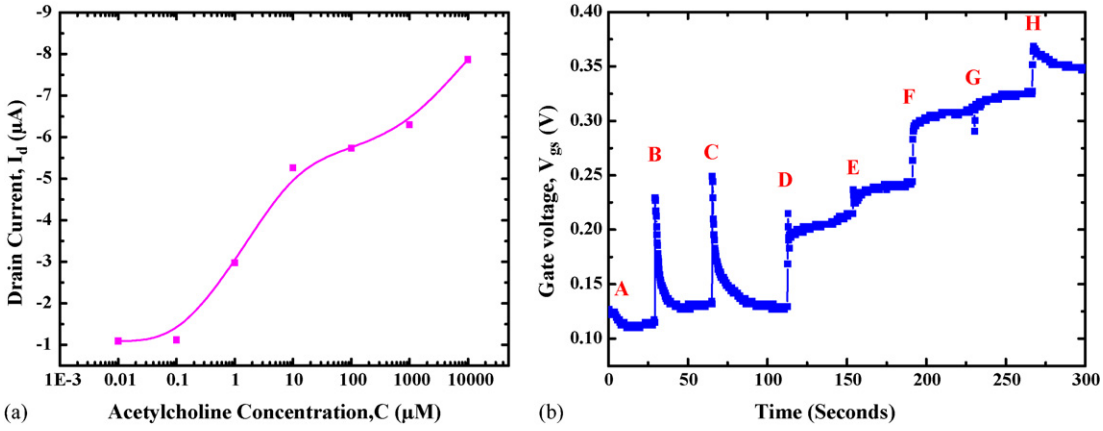


Fig. 5. (a) The sensitivity of the PANI ISFET on silicon substrate for sensing acetylcholine concentration. (b) The response time of the PANI ISFET at different concentration of acetylcholine: (A) DI water, (B) 10 nM, (C) 100 nM, (D) 1 μM, (E) 10 μM, (F) 100 μM, (G) 1 mM, and (H) 10 mM.

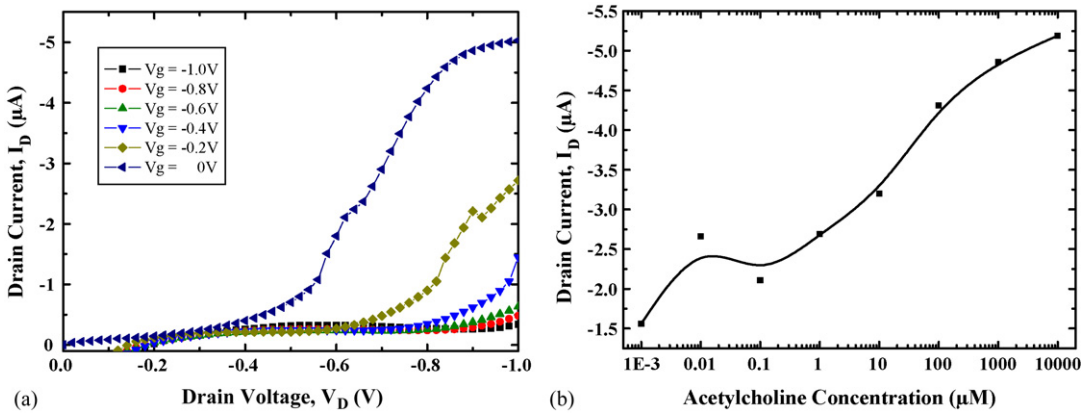


Fig. 6. (a) The output characteristic of the PANI ISFET on plastic substrate in 10 mM acetylcholine solution. (b) The sensitivity of the PANI ISFET on plastic substrate for sensing acetylcholine concentration.

and resolution. The reason might be that polymer/nanoparticle thin films can be LBL self-assembled better on silicon substrate than plastic substrate.

4. Conclusions

In this paper, polyaniline ISFET based on the low-cost layer-by-layer nano self-assembly was successfully fabricated and used for the acetylcholine biosensing. Both silicon wafer and flexible transparency have been used as the substrates. Acetylcholine concentration down to 1 μM could be detected use the self-assembled biosensors. The ISFET exhibited high performance, operating at very low voltages with a high mobility. The results show promise for a large scale logic circuit integration of biosensors and biomedical systems. Further work is ongoing to improve the sensitivity of the ISFET for acetylcholine sensing such that it could detect acetylcholine down to 10 nM.

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Biographies

Yi Liu received his BS degree in telecommunication engineering from Beijing University of Posts and Telecommunications (Beijing, China) in July 1998, and his PhD degree in mechanical engineering from University of Minnesota at twin cities (Minnesota, US) in May 2006. Recently, he has joined in the vacuum process department at Seagate Technology Inc.

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