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Characterization of Functional Biointerface on Silicon Nanowire MOSFET

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***Abstract*—Biointerface between biological organisms and electronic devices has attracted a lot of attention since a biocompatible and functional interface can revolutionize medical applications of bioelectronics. Here, we used 3-aminopropyl trimethoxysilane (APTMS) self-assembled monolayer (SAM) to modify the surface of nanowire-based metal-oxide-semiconductor field-effect transistors (NW-MOSFETs) for pH sensing and later creation of biointerface. Electrical measurement was utilized to first verify the sensing response of unmodified NW-MOSFETs and then examine pH sensing on APTMS modified NW-MOSFETs. A biointerface was then created by immobilizing polylysine, either poly-D-lysine (PDL) or poly-L-lysine (PLL), on APTMS modified NW-MOSFETs. This biointerface was characterized by electron spectroscopy for chemical analysis (ESCA), cell biocompatibility, and fluorescent images. The results of ESCA verified the amide bonding (CONH) between polylysine and APTMS modified surface. After PC12 cultured on polylysine-APTMS modified area, highly selective areas for cell growth were observed by fluorescent microscope. Analysis and improvement of selectively cell-growth biointerface on the NW-MOSFETs gave us an insight into future development of neuronal biosensors.**

I. INTRODUCTION

METAL-oxide-semiconductor field-effect transistors (MOSFETs) have been developed by using advanced micro and nano electro-mechanical systems (M/NEMS) technology. The sensing mechanism of MOSFETs is the external localized field influences carrier distribution in the near surface region of a sensing area. Recently, there are various nanowire-based FETs have been explored in many biomedical aspects because of their high selectivity, extreme sensitivity, rapid response, and potential for integration into full electronic on-chip systems for high-throughput biological analysis [1]-[5].

Techniques of surface modification have been applied to functionalize FET for the specific detections of ions [6], DNA [7], proteins [1][5], and viruses [8]. In most circumstances, the surface modification or functionalization is to introduce functional group; 3-aminopropyl trimethoxysilane (APTMS) has been one of the useful molecules for creating a self-assembled monolayer (SAM) on the surface of silicon-based electronic devices [1][4]. Biointerface between biological organisms and electronic devices can be easily constructed by immobilizing biomolecules on APTMS-SAM [1]. The sensing response and sensitivity of FET are subjected to several factors, such as an appropriate technique to modify and functionalize the surface, Debye length [1][9], and the efficient amount of corresponding molecules, etc. However, there is a lack of studies in investigating those factors except Debye length [1][9].

In this work, the APTMS-SAM was formed on silicon oxide surface of phosphorus-doped n-type nanowires. The application of APTMS modified nanowire-based MOSFETs (NW-MOSFETs) for quantitatively electrical measurements were recorded for elucidating the suitable modification would have great impact on the sensing response and sensitivity. The amino ($-NH_2$) groups of APTMS contributed a functional interface to immobilize polylysine molecules, either poly-D-lysine (PDL) or poly-L-lysine (PLL). Polylysine modified area was characterized by electron spectroscopy for chemical analysis (ESCA), fluorescent observation, and cell culture test.

II. MATERIALS AND METHOS

A. PH sensing experiment

Silicon oxide was modified with APTMS (Sigma) to provide terminal amino groups on the nanowires. First, the chip was cleaned in ethanol for 10 min to remove organic contaminants on surface of nanowires. The nanowire was then modified with 1% ethanolic solution of APTMS for 1 hr. The sample was rinsed with ethanol for several times and then heated in ethanol in an oven at 60°C for 5 min. The APTMS-SAM modified NW-MOSFETs was ready for pH sensing by using electrical measurement and for construction of biointerface by further immobilizing PDL or PLL. Different buffered pH solutions (including pH 4, pH 7.4, pH 9, and pH 11) were individually introduced to unmodified or modified MOSFETs. DI water was used to rinse the surface of nanowires before next pH solutions sensing. The biointerface was created by immersing APTMS modified NW-MOSFETs in 100 μg/ml PDL (Sigma) phosphate buffered saline (PBS) containing N-Hydroxysuccinimide and ethyl(dimethylaminopropyl) carbodiimide at pH 7.4 overnight.

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B. Surface Characterization

ESCA was applied to analyze the elemental composition and characterize specific chemical structure in each-step modification of NW-MOSFETs. The ESCA spectra were acquired with a VG Scientific Microlab 310F, a micro-focus non-monochromatic aluminum anode (Al (Kα) 1486.6 eV), and a Concentric Hemispherical Analyzer. The C1s and N1s core level spectra were measured and fitted by using Voigt peak profiles and a Shirley background [1][10] together with a database for semiconductor [11] and organic polymers [12].

C. Fluorescent observation

The fluorescent images were observed and photographed using a phase-contrast upright microscope (Nikon, ECLIPSE 80i) equipped with fluorescent optics and an intensified charged coupled device camera. In order to verify the areas of selective biointerface, 100 μg/ml PLL labeled with fluorescein isothiocyanate (PLL-FITC) (Sigma) was immobilized on APTMS modified surface by using the same procedure as PDL immobilization. For fluorescent observation of cell staining, samples were stained by the following procedure of cell staining and examined by the fluorescent microscope.

D. Electrical measurement

The variations of voltage signal of unmodified and APTMS-SAM modified NW-MOSFETs, were independently measured in the subthreshold regime by using an Agilent 34401A 6 ½ Digit Multimeter under a constant 1.5 V ac voltage (DDS function Generator GW INSTEK SFG-2020) at 1 kHz [13][14].

E. Cell culture examination

After PDL or PLL-FITC was immobilized on APTMS modified NW-MOSFETs and substrate, PC12 cells, rat adrenal gland pheochromocytoma, were seeded onto those samples. PC12 cells were maintained in RPMI1640 medium (Biowest) with 10% horse serum (Gibco), 5% fetal bovine serum (Gibco), 100 μg/ml P/S (Biowest), and 29.2 μg/ml glutamine (Biowest). Cells were then cultured at 37 °C in a humidified atmosphere with 5% CO_2. PC12 cells further differentiated into neuron-like cells after treatment with 100 ng/ml β-NGF (Sigma) in serum-free RPMI1640 medium. For preparation of cell staining, cells were fixed on day 3 of culture with 4% paraformaldehyde (Sigma) for 20 min at RT. Subsequently, the cells were washed with pH 7.4 PBS and treated with 0.1% Triton X-100 (Sigma). Samples were then stained with rhodamine–phalloidin (1:100, Molecular Probes) in pH 7.4 PBS for 45 min. Samples were washed twice with pH 7.4 PBS and examined by fluorescent microscope.

III. RESULT AND DISCUSSION

Electrical measurement was used to verify that the adequate modification would have great impact on the sensing response and sensitivity of NW-MOSFETs in our study. First, air and DI water was used to evaluate the sensing response of unmodified NW-MOSFETs. When air or DI water reached nanowire, a significant and repeatable change of electrical signal was observed at 1 kHz (blue line in figure 1) in contrast with the random and irregular signal at 20 Hz (red line in figure 1). In addition, we found the average of sensing response in unmodified NW-MOSFETs was around 1.065 sec. The results of electrical measurement regarding pH sensing using APTMS-SAM modified and unmodified nanowires were shown in figure 2. In contrast to unmodified nanowires (blue line in figure 2), the signal of APTMS-SAM modified NW-MOSFETs in figure 2 (red line) was enhanced and shown APTMS-SAM did not deteriorate the sensing signal of NW-MOSFETs. We found the average of sensing response in APTMS-SAM modified NW-MOSFETs was around 1.248 sec. The amino group promisingly improved the sensitivity of nanowires in pH sensing. It is worth mentioning that the voltage would return back to the initial level after each step of measurement on either unmodified or modified NW-MOSFETs. By contrast to other studies [1][4][9][15], our results of electrical measurements also show the stability of unmodified NW-MOSFETs and APTMS-SAM modified NW-MOSFETs are still functionable and also sensitive to the external environmental changes.

To examine each-step of modification for biointerface on NW-MOSFETs, we identified the specific chemical structure by ESCA. Figure 3 and Figure 4 are represented C1s and N1s, respectively. In C1s spectra, the specific peaks are observed at 283.9, 285.0, and 286.8 eV corresponding, respectively, to the silicon carbide (Si-C), carbon-carbon (C-C), and amine (C-NH_2) groups of APTMS. After PDL immobilized on APTMS, a significant peak occurred at 288.5 eV (amide, CONH group). In accordance with the C1s spectra, the N1s spectra also showed spectral amide-feature at 398.9 eV. Since the APTMS can provide a functional amino group (NH_2) to bridge other biomolecules with carboxy group (COOH), a biointerface would be formed by amide bond on the surface of NW-MOSFETs. Therefore, we concluded those spectral features to elucidate our engineered biointerface on NW-MOSFETs in Figure 5. The SAMs modified surface of nanowires offered a reliable interface to immobilize polylysine through covalent amide bond. PDL and PLL, differ in the steric configuration of the molecule, are amino acids used to enhance cell attachment on plastic and glass surfaces [16]. It means the mechanisms of immobilizing PDL and FITC-PLL on APTMS modified NW-MOSFETs were the same. That is to say, PDL and PLL could be immobilized by amide bond on APTMS modified NW-MOSFET.

In order to validate and qualify the areas of biointerface on APTMS modified NW-MOSFET, PLL-FITC was immobilized on APTMS modified surface as the same procedure of immobilizing PDL. The APTMS-SAMs labeled PLL-FITC was observed by fluorescent upright microscope. The FITC was observed as green fluorescence in Figure 6. Black areas were silicon nitride material. Therefore, the selectively modified areas existed on the NW-MOSFETs were clarified by these green ordered-square patterns. Furthermore, after PC12 cells seeded on PLL-FITC immobilized samples, Figure 7 showed red-color cells grew on the green areas and connected each other. In addition, PC12 cells also grew very well on PDL immobilized samples

(data not shown). While both PDL and PLL are widely used, PDL may be preferred for some cell types and applications because PDL is not broken down by the proteases released by cells in culture [17]. For that matter, it is stable to employ PDL in our engineered biointerface. These phenomena also explained our engineered biointerface possesses cell compatibility. According to the results of ESCA and cell culture assays, we conjectured there is an electrostatic interaction between cells and our engineered biointerface in Figure 5. The amine groups in PDL or PLL of our biointerface were easy to be protonized and become NH_3^+ groups in the physiologic saline solution. Therefore, the positively charged PDL or PLL of our interface could attract PC12 cells because of negatively charged cell membrane. Here, we have successfully engineered a functional biointerface for cell growth on NW-MOSFETs. In order to further develop neuronal biosensors, electrophysiological measurements using modified NW-MOSFETs need to be taken into account in the future.

IV. Conclusion

Analysis and improvement of selectively cell-growth biointerace on the NW-MOSFETs gave us an insight into future development of neuronal biosensors. Electrical measurement showed that the APTMS modified nanowires were still functionable and also sensitive to the external environmental changes. ESCA spectra showed the chemical structures of our engineered biointerface on the PDL-existing APTMS modified NW-MOSFETs. Moreover, the selective modified areas of our engineered biointerface provided a cell biocompatibility. We believe that biointerface provides a biocompatible and functional interface and also revolutionizes medical applications of bioelectronics in the future.

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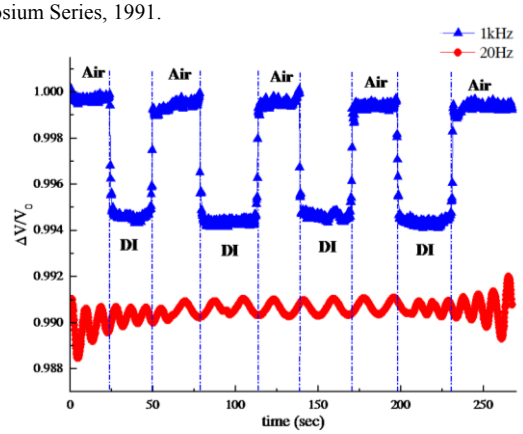

Figure 1 Real-time sensing responses of unmodified NW-MOSFET were recorded at 20Hz (red line) and 1kHz (blue line).

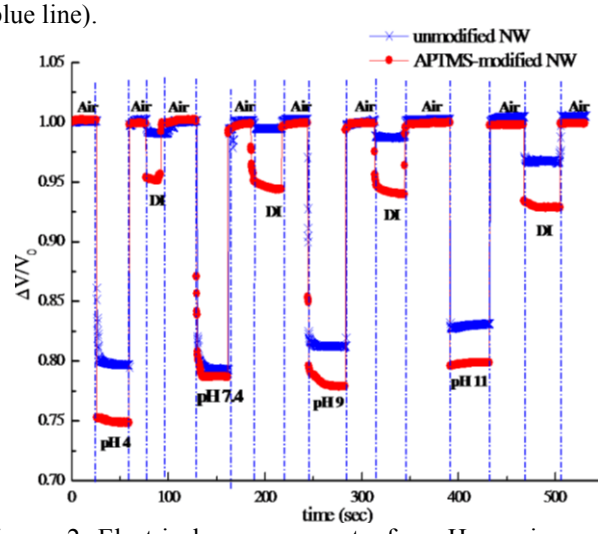

Figure 2 Electrical measurements for pH sensing were recorded at 1 kHz by using unmodified nanowires (blue line) and APTMS-modified (red line) NW-MOSFETs.

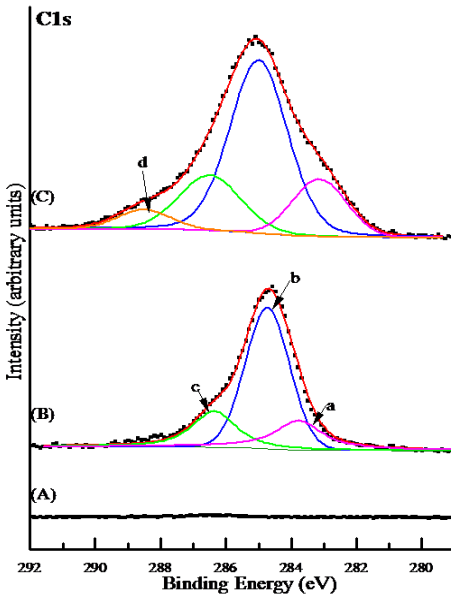

Figure 3 C1s ESCA spectra obtained from each-step modification of silicon nanowire. (A) Unmodified silicon nanowire, (B) silicon nanowire modified with APTMS, (C) silicon nanowire modified with PDL-APTMS. The assignments of the individual spectral components, correlated with the element C's functionabilities, were marked on the spectrum. The deconvoluted peaks were fitted with the following binding energies: (a) 283.9 (Si-C), (b) 285.0 (C-C), (c) 286.8 (C-NH_2) ,and (d) 288.5 eV (CONH).

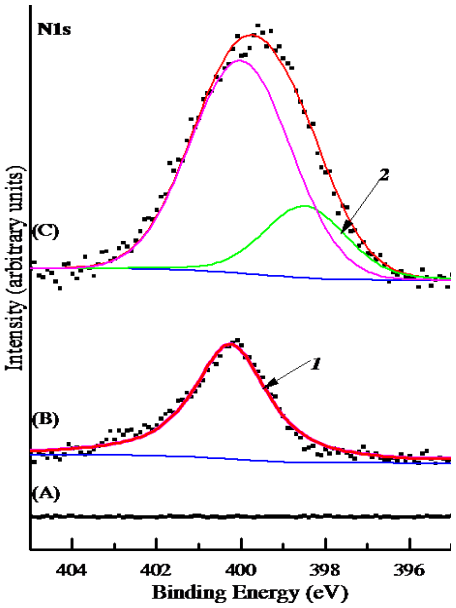

Figure 4 N1s ESCA spectra obtained from obtained from each-step modification of silicon nanowire. (A) unmodified silicon nanowire, (B) silicon nanowire modified with APTMS, (C) silicon nanowire modified with PDL-APTMS. The assignments of the individual spectral components, correlated with the element N's functionabilities, were marked on the spectrum. The deconvoluted peaks were fitted with the following binding energies: (1) 399.8 (C-NH_2) and (2) 398.9 eV (CONH).

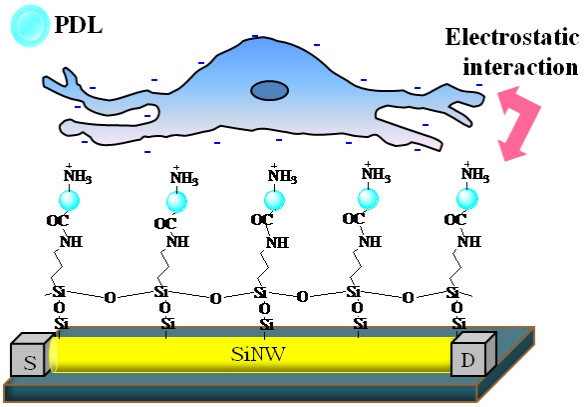

Figure 5 The scheme of our engineered biointerface on NW-MOSFET. The amine groups of our biointerface were easy to be protonized and become NH_3^+ groups in the physiologic saline solution. The positively charged interface could attract PC12 cells by an electrostatic interaction.

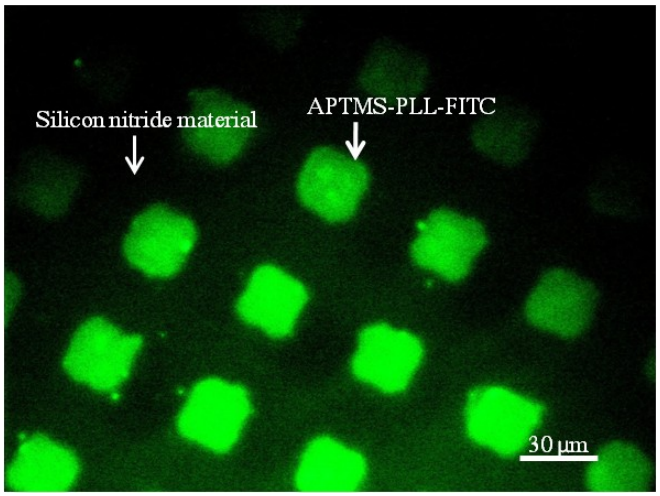

Figure 6 PLL-FITC on APTMS modified silicon NW-MOSFETs. Parallel nanowires MOSFETs were beneath these green ordered-square patterns.

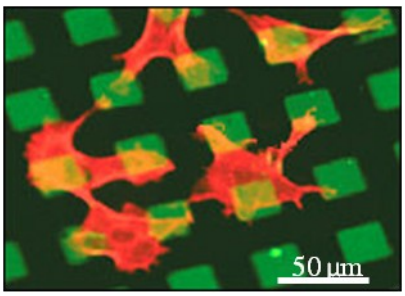

Figure 7 PC12 cells (red color) cultured on APTMS modified silicon NW-MOSFETs with PLL-FITC immobilization.