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Study of TiO_2 NWs-based FET immunosensor: effect of surface immobilization methods

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Abstract

Immobilization of biomolecule on biosensor surface is an important issue for develop higher sensitivity ***immunosensors***. Recognition elements can be modified on material surface through chemical conjugate, physical adsorption and, polymer entrapment etc. Although most of these methods are well established, none of them can perform both *in situ immobilization* of biomolecues and be easily established. In this study, anti-rabbit IgG (1^oAb) is encapsulated by a cyclic-voltammetry for *electropolymerization* of *pyrrole propylic acid* (Pa) on self-designed TiO_2 NWs *field effect transistor*. A better specificity, selectivity and sensitivity can be achieved at nano-gram level (R^2= 0.903) when compared with 3-aminopropyltrimethoxysilane (APTMS).

Key words: *in situ* immobilization, electropolymerization, pyrrole propylic acid, field effect transistor

Introductions

Over the past decade, field effect transistors (FETs) constructed by one-dimensional (1-D) nanomaterial have been widely introduced to biosensing research and applications owing to its high sensitivity and specificity properties. Most important part of all nanostructure-based materials for biosensors is their ability of specific antibody recoganetions. Many chemical- and physical-based methods, such as silane linking or polymer entrapment, are well-established protocols for immobilizing biomolecules on matrices [1-3] and related technologies have become the standard method [4]. Unfortunately, device sensitivity may decrease when non target area is polluted via physical adsorptions of proteins. In this research, we compared two methods for encapsulation of biomolecules on biosensors using 3-aminopropyltrimethoxysilane (APTMS) and polypyrrole propylic acid (Pa). Electrochemical polymerization of a low-conductivity polymer Pa (PPa) [1] for the encapsulation of biomolecules on TiO_2-nanowire (NW) FET immunosensor is discussed. The electrical analysis and the energy dispersive spectrum were recorded for investigation of PPa and 1^oAb on a composite film. The specificity, selectivity and sensitivity of the biosensor were analyzed in order to determine the immunoreaction of PPa/1^oAb immobilized TiO_2-NW FET immunosensors.

Materials and Methods

TiO_2 NWs was made of simple hydrothermal synthesis method followed by spin-coated on the gold microelectrode with designed gap of 1 μm [4]. Mixture solution contains 15 mM of polypyrrole propylic acid (Pa) (supported by C. M. Li [1]) and 100 μg/mL primary antibody (Anti-rabbit IgG, 1^oAb) was added into 1 mL of 10 mM phosphate buffered saline (PBS) solution and then electrochemical polymerized to form a composite film on patterned NWs. Electrochemical polymerization was performed by using cyclic voltammetry method at a voltage ranging from 0 to 0.76 V vs. Ag/AgCl for 20 cycles, scan rate of 0.1 V/sec, and with a Pt counter electrode [1].

Characterization of antibody immobilized-TiO_2 NWs immunosensor shows good affinity for rabbit IgG (secondary antibody, 2^oAb) detection which performed on our designed device. The surface properties changes on the designed immunosensor during the preparation processes of NWs, PPa (PPa/TiO_2 NWs), anti-rabbit IgG (1^oAb/PPa/TiO_2 NWs immunosensor), and the specific adsorption of rabbit IgG (2^oAb-1^oAb/PPa/TiO_2 NWs immunosensor) were recorded and expressed by the changes of current-voltage.

Results and Discussions

Diameters of prepared TiO_2 NWs are calculated around 250 nm to 400 nm from SEM image, as showed in Figure 1.

Synthesis TiO_2 NW presents anatase structure as three characteristic peaks stand at 395, 514 and 638 cm^{-1} analyzed under Raman spectroscopy with 532 nm laser (see Fig.2). Mixture contains synthesis NWs was then spin-coated on the Au/Ti microelectrodes with the designed gap of 1 μm.

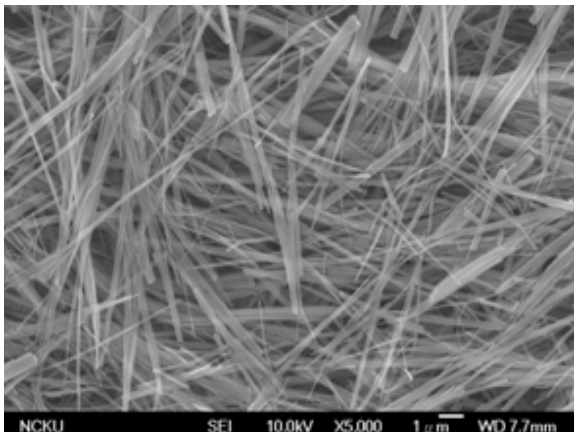

Fig. 1. SEM image of TiO_2 NWs on designed electrode

Experiment setup of the electrochemical polymerization system and the structure of conduction polymer (pyrrole propylic acid) before and after polymerization were showed in Fig. 3(a). Cyclic voltametry was applied for *in situ* electrochemical polymerization of pa-co-1 °Ab (100 μg/mL of 1°Ab with 15 mM Pa in a 10 mM PBS) on the surface of NWs. Operation conditions for cyclic voltammetry was set at 0~0.76 V with scan rate 0.1 V/sec for 20 cycles. As illustrated in the inset figures, antibody can be encapsulated around the surface of NWs during the polymerization process in which PPa was polymerized from Pa monomer.

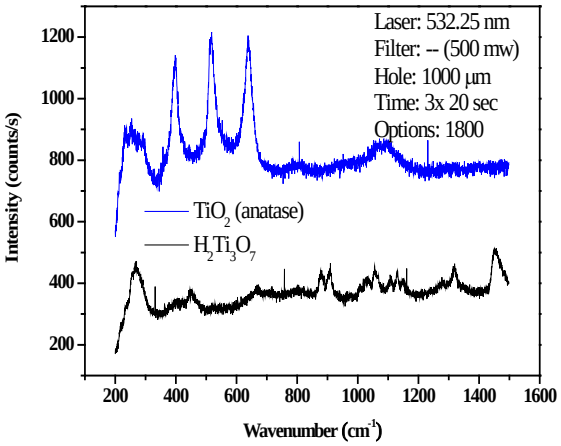

Fig. 2. Raman spectra of prepared TiO_2 NW. Anatase structure can be characterized by identified peaks at 395, 514 and 638 cm^{-1}.

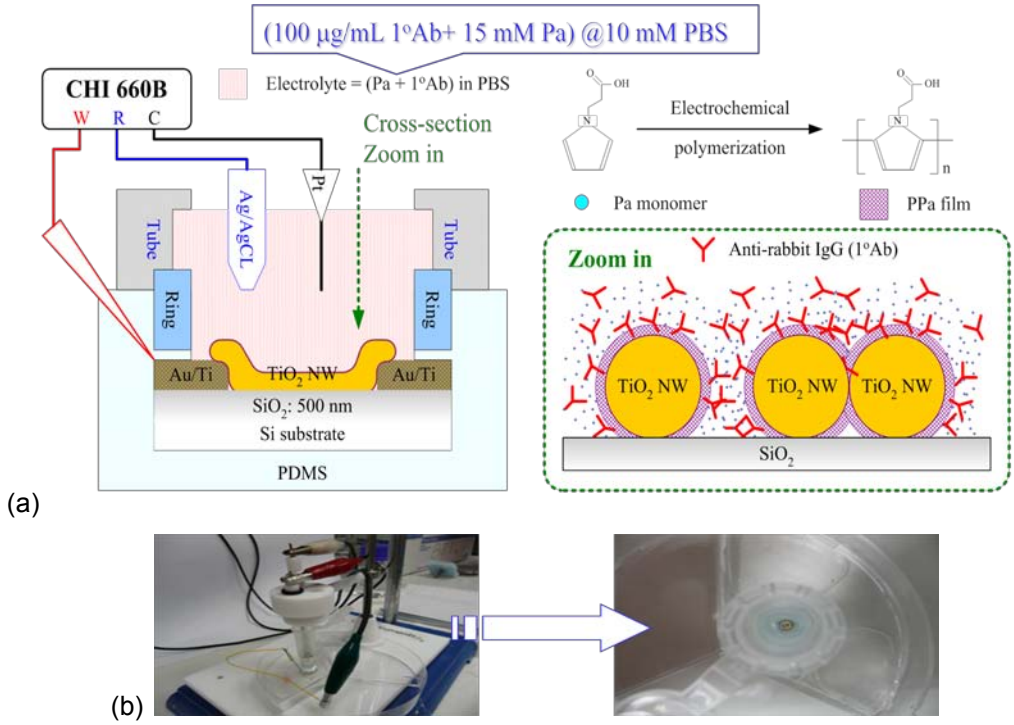

Fig. 3. Schematic diagram (a) and photos(b) of electrochemical polymerization system setup. Mixture of electrolytes was prepared by adding 100 μg/mL of 1°Ab with 15 mM Pa in a 10 mM PBS. Operation conditions for cyclic voltammetry was set at 0~0.76 V with scan rate 0.1 V/sec for 20 cycles. Antibody can be encapsulated around the surface of NWs during the polymerization process which PPa was polymerized from Pa monomer.

The target biomolecules can be entrapped via electrochemical polymerization of PPa on the designed TiO_2 NWs immunosensor while the APTMS group was stand-coated on the whole area of the device surface. Figure 4 indicated the result of DES analysis on NWs surface. Here blue and red color represent the distribution of Ti and C elements, respectively.

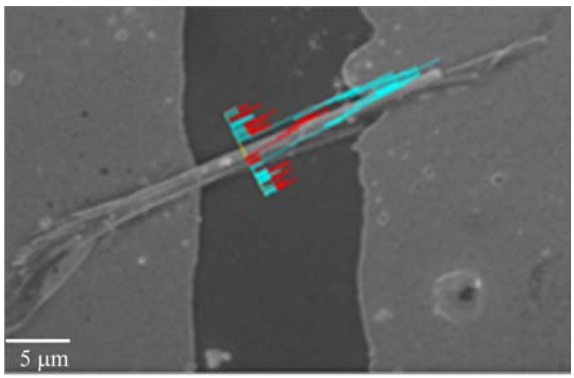

Fig. 4. DES analysis of NWs. Blue and red color represent the element contents of Ti and C, respectively.

Measured current changed before and after polymerization, and after affinity reacted with 11.9 ng/mL of 2^oAb (V_{DS} = 5 V), as response curves showed in Fig. 5(a) and 5(b). As can be seen clearly, when polymerization mixture contains no 1^o Ab, there are no current changes when target 2^oAb was added.

Response curve of I_D-2^oAb concentration presents linear decrease tendency with the increase of target 2^oAb concentration (Fig. 6). In contrast, immunoresponse of polymerized PPa-based TiO_2-NW-immunosensor group showed a better sensitivity with linear region (V_{DS} = 5 V) at 2^oAb concentrations lower than 5.95 ng/mL when compared with APTMS modified group (Fig. 6a). Below two schematics illustrated the surface modification difference between PPa and APTMS on the TiO_2 NWs-based immunosensor device.

Our results shows higher similarity to that of previous literatures, the cross section of depletion region, near the TiO_2 NWs surface might be increased while the concentration of adsorbed target 2^oAb increased; otherwise the current flow in the back of substrate was decreased significantly under bias-free status.

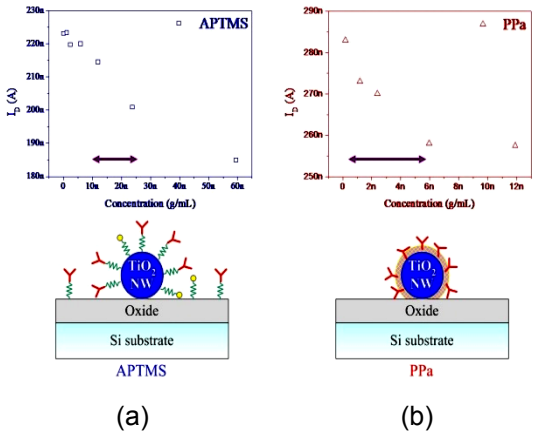

Fig. 6. Response curve of I_D under various concentrations of 2^oAb and the schematic diagrams of two different modification methods. Biomolecule immobilized by APTMS (a)and via PPa (b).

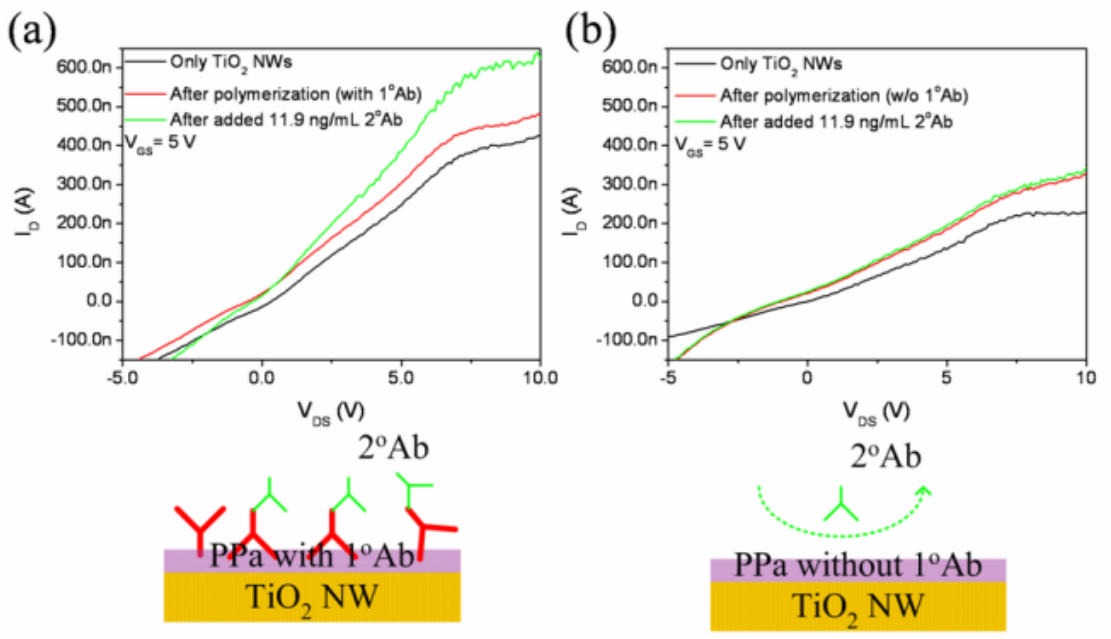

Fig. 5. Measured current changed before and after polymerization, and after affinity reacted with 11.9 ng/mL of 2^oAb (V_{DS} = 5 V). Biomolecule immobilized by APTMS (a)and via PPa (b).

Conclusion

Encapsulation of biomolecules via the electropolymerization of low conductivity PPa on TiO_2 NWs immunosensor achieves a better sensitivity than that of using APTMS. Detection limitation of the target rabbit IgG can as lower as to nano-gram level (R^2= 0.903).

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