A pH sensor based on electric properties of nanotubes on a glass substrate

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Abstract We fabricated a pH-sensitive device on a glass substrate based on properties of carbon nanotubes. Nanotubes were immobilized specifically on chemically modified areas on a substrate followed by deposition of metallic source and drain electrodes on the area. Some nanotubes connected the source and drain electrodes. A top gate electrode was fabricated on an insulating layer of silane coupling agent on the nanotube. The device showed properties of an *n*-type field effect transistor when a potential was applied to the nanotube from the top gate electrode. Before fabrication of the insulating layer, the device showed that the *p*-type field effect transistor and the current through the source and drain electrodes depend on the buffer pH. The current increases with decreasing pH of the CNT solution. This device, which can detect pH, is applicable for use as a biosensor through modification of the CNT surface.

Keywords Carbon nanotube · Field effect transistor ·
pH · Glass · Immobilization

Introduction

Because single molecule research sometimes contributes to mechanisms of biomolecular interactions, some methods

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have been developed to observe or manipulate single molecules in the last decade: laser tweezers, scanning probe microscopy, optical microscopy, and so on. The combination of these methods yields more information about the mechanism of interactions. Nevertheless, methods for observing a single molecule reaction are not numerous: they require ultra-sensitivity and a very small detection area.

Considering those requirements, a nanotube-FET device is a candidate as an observation method for single-molecule level interaction. The device can detect a single biomolecule interaction and the nanotube device can be miniaturized to the sub-micrometer scale. Many biomolecules interactions including single-virus binding, antigen-antibody bindings, streptavidin-biotin binding, DNA-hybridization, pH, and some gases have been detected using this nanotube-based device [1–8]. Consequently, the combination of nanotube-FET devices with other apparatus might contribute to research work in many fields. For example, a combination with a total internal reflection system on an optical microscope (TIRF) and a CNT-FET device on glass might elicit additional information about the interaction processes of proteins, DNA-hybridization, antigen-antibody, and many biomolecular reactions on a single-molecule scale.

Therefore, advantages of the device on a transparent substrate are not only those for commercial products such as memory, circuits, and chemical sensors, but also for investigation of reactions or interactions of biomolecules on the scale of single molecules. Recently, a nanotube-based FET device on an optically transparent substrate, such as sapphire glass, was investigated [9]. Unfortunately, some limitations exist for the substrate thickness for combination with commercial TIRF systems. A sapphire substrate is unsuitable for commercial TIRF systems. Our

objective is to fabricate a nanotube device on a cover glass and to investigate its applications.

Regarding the fabrication process of the nanotube FET sensor, chemical vapor deposition (CVD) method has been shown to improve the yield and quality of the CNT-FET device. This method is useful to control not only the type of nanotube (single-walled (SW) CNT or multi-walled CNT) but also its growth length and density. One disadvantage of the method is that the substrate must be heated to 900 °C during CNT growth. Therefore, the use of the method is limited according to the substrate used. For example, we were unable to prepare CNT sensors on a glass substrate using CVD.

Before the CVD method was developed, the device had been fabricated by adding the CNT dispersion on the source and drain electrodes. The improved method is the controlled localization method of CNT on a silanized silicon oxide patterned substrate via the amino groups [10, 11]. Using this method, the limit for the choice of the type of substrate is reduced because the substrate requires no heating above 900 °C. However, the affinity of the amino groups to the CNT was insufficiently strong. Therefore, the CNT is sometimes absorbed to the surface nonspecifically. Auvray et al. reported an effective method to localize CNTs on a thin layer of 3-aminopropyltriethoxysilane (APS) patterned substrate by selecting an organic solvent [12]. They also studied the effect of doping by APS on the electric properties of the CNT. In their method, primary amino groups of APS on a silicon substrate were protected first; then the amino group was recovered by light exposure. Recovered amino groups are useful to immobilize nanotubes in the organic solvent; the protected area inhibits nonspecific binding of the nanotubes. The process of immobilization is somewhat complex and a proton releasable photo-resist is required. Recently, Lee et al. fabricated a CNT-FET device on various substrates using a bare SWCNT dispersion in an organic solvent that is sometimes difficult to handle [13]. For example, nonspecific binding to the bare substrate increases even though non-specific binding to a nonpolar layer such as octadecyltrichlorosilane (ODT) is very low. In their study, CNTs are immobilized specifically on a bare substrate and the ODT layer is used as a non-binding area. One attendant problem might be immobilization of biomolecules on the CNT-FET. For immobilization of biomolecules on the device surface, ODT might be a problem for immobilization of the biomolecules because of their lack of chemical reactivity and for non-specific adsorption of biomolecules. Direct modification of the CNT surface sometimes showed no good reproducibility. In their case, a reactive group must be placed on the SWCNT or on the device. For those reasons, an improved method is still required for CNT-FET devices for biomolecule sensors and chemical sensors.

Recently, we found that SWCNT dispersion, which is treated using a mixture of acids and hydroxyperoxide, showed good stability. Furthermore, its aggregation behavior is controllable by changing pH. This SWCNT can be immobilized specifically on the APS layer in water. For this study, we fabricated a nanotube FET device on a cover glass after fabricating a sol-gel layer of APS at the planned site of source and drain electrodes on the glass substrate because the sol-gel layer immobilized nanotubes more efficiently onto the planned site than on the thin layer of APS. After deposition of Cr/Au to the site, we investigated the properties of the CNT device on the cover glass. We also discussed the application of the device as a chemical sensor.

Materials and methods

From Sigma-Aldrich Corp., Japan, 3-aminopropyltriethoxysilane (APS), disodium hydrogen phosphate, and dihydrogen sodium phosphate were purchased. Single-wall carbon nanotubes were purchased from Carbon Nanotechnologies Inc., USA. Sulfuric acid, nitric acid, hydrogen peroxide, N,N-dimethlyformamide (DMF), and ethanol were obtained from Kanto Chemical Co. Inc., Japan. Cover glass was purchased from Matsunami Glass Ind., Ltd., Japan. A photoresist (OFPR-800) was purchased from Tokyo Ohka Kogyo Co., Ltd., Japan.

CNT immobilization on glass

Fabrication of a sol-gel layer of APS on the cover glass

A schematic model of the fabrication process of a CNT device is shown in Fig. 1. First, the OFPR-800 film was spin-coated on the glass substrate using a spincoater. Pairs of areas were fabricated in the PMMA film using photolithography and ordered pairs of electrode patterns were obtained (Fig. 1a). A 1% APS solution was dropped on the substrate. It was heated at 45 °C for 15 min until 70% of the solution had evaporated. After removing the solution by nitrogen gas blowing, the substrate was heated to 110 °C for 25 min. The substrate was immersed in pure water for 3–8 min with gentle shaking. Then the substrate was put in the DMF solution to remove the PMMA layer (Fig. 1b). Thickness of the sol-gel APS layer was 50–200 nm, depending on the water immersion time.

CNT immobilization

With a mixture of H_2SO_4 and HNO_3, 0.5 mg of SWCNTs were washed. The solution was centrifuged, and the residue was suspended in a mixture of H_2SO_4, HNO_3 and H_2O_2 for

Fig. 1 Schematic model of the fabrication process of CNT-FET on a cover-glass substrate
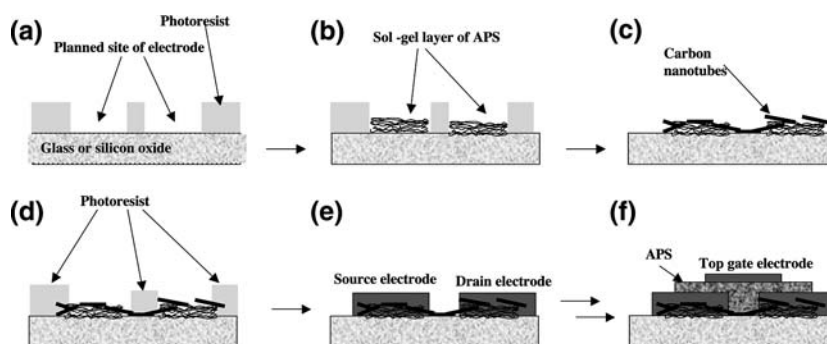
1 h with ultra-sonication. The obtained black solution was diluted with water and subjected to dialysis with distilled water to obtain a neutral pH for the solution. The CNT solution, which was resonicated immediately before use, was placed on the patterned substrate at room temperature for 1 min to adsorb the CNT on the APS layer only (Fig. 1c). The substrate was washed with water. The topography of the CNT-immobilized areas was observed using AC-mode atomic force microscopy (AFM).

Deposition of source and drain metallic electrodes

The patterns used for formation of metallic electrodes were fabricated on CNT-immobilized areas using a similar process to that used for formation of areas for immobilization. The electrode was one size larger than the APS layer to cover the layer (Fig. 1d). As metallic electrodes, a 30-nm-thick Cr film and a 50-nm-thick Au film were deposited using evaporation deposition (Fig. 1e).

Deposition of gate metallic electrodes

The patterns used for formation of gate metallic electrodes were fabricated using a similar method of fabricating source and drain electrodes, except that the APS sol-gel layer was formed on the CNT and source and drain electrodes (Fig. 1f). After removing the photoresist, the substrate was heated at 185 °C for 1 min to dehydrate and condense the APS layer.

Topographic images

The topographic images of samples were observed using an atomic force microscope (MFP-3D; Asylum Research, USA) and a silicon AFM tip (OCL-400; Olympus Optical Co. Ltd., Japan). All topographies were observed in air at room temperature in the AC mode.

I–V measurements and pH measurement

To examine the CNT properties, I_{ds} was measured in air by applying –2 to +4 V to the top gate built on an APS layer

on the CNTs. In addition, I_{ds} was plotted as a function of the potential of the top gate (V_{gate}) between the source and drain (I_{ds}–V_{gate} curve). The potential between the source and drain electrode was +0.1 V or –0.1 V. The I_{ds}–V_{ds} curves were obtained when the gate potential was varied from –2 V to 4 V.

I_{ds}–$V_{liquid\ gate}$ curves were measured in 10 mM phosphate buffer with 100 mM KCl at pH 7. An Ag/AgCl electrode was used as a gate. The potential between the source and drain electrode was +0.1 V or –0.1 V. The I_{ds}–$V_{liquid\ gate}$ curves were obtained when the gate potential was varied from –0.55 V to –0.15 V.

Effects of the pH on I_{ds} were measured dropping various pH of 10 mM phosphate buffer on the CNT device: 20 micro L of the buffer was dropped on the CNT and the Ag/AgCl electrode was used as a reference electrode. The distance between the CNT device surface and the electrode was less than 1 mm. As for controlling the solution pH, 10 micro L of the solution was suctioned using a pipette; then 10 micro L of the other pH solution was added. The pH of the suctioned solution was measured using a commercial MOS-FET-based pH meter. During measurements, 10 mL of water in dishes was set near the apparatus to prevent evaporation of the buffer. The potential between the source and drain electrode was 0.1 V. A semiconductor characterization system (4155C; Agilent Technologies, Inc.) was used to measure the I–V curves at room temperature.

Results and discussion

Fabrication of CNT-FET on the cover glass substrate

In this study, SWCNT were washed and modified using a mixture of sulfuric acid, nitric acid, and hydrogen peroxide. The SWCNTs, when washed using a strong acid such as sulfuric acid, display carboxylic acid at the edge and SWCNT defects. Amorphous carbons, whose surfaces have carboxylic acid groups, sometimes coat the surface of SWCNTs. These SWCNTs can be immobilized specifically on the APS layer in water because of electrostatic interactions between negatively charged SWCNTs caused by

carboxylic acid groups and the positively charged APS layer. Recently, we found that the sol-gel layer of APS was able to bind bundles of SWCNTs specifically in an aqueous condition. The surface topography of the cover glass was measured using AFM after immobilization of the bundles on the APS sol-gel layer; it is shown in Fig. 2. As shown in Fig. 2, bundles of the SWCNT bound specifically to the sol-gel layer. Some were located between the planned area of the source and drain electrodes. The APS layer thickness was controlled by controlling the immersion time in pure water before the lift off process using DMF (data not shown). For a thick layer of greater than 100-nm thickness, the number of SWCNT bundles on the layer increased and some nanotubes ran off the edge. For a thin layer, e.g., a less than 10-nm-thick layer, their number decreased and almost all nanotubes were immobilized on the layer. We applied the phenomena of running off the bundles from the edge of the APS layer for immobilizing SWCNTs between the planned electrodes areas. In this study, we controlled the layer thickness as 50–400 nm so that the SWCNT was able to connect to the source and drain area after electrode deposition. When washing the substrate using pure water after immobilization of SWCNT on it, the direction of the nitrogen gas flow for drying the surface was controlled from the source to the drain or the drain to source electrode. Using successive processing, efficiency for localizing the bundles between the electrodes was improved over that of using a single layer of APS.

After deposition of the source and drain electrodes, the top gate was fabricated on a 500-nm-thick APS layer on nanotubes and source and drain electrodes (Fig. 1f). We used APS as an insulator. We used only SWCNT devices for which the current from the top gate to source or drain electrodes was negligible: less than 10 nA, for I_{ds}. The I_{ds} was measured in air when V_{gate} was applied as –2–+4 V. The I_{ds}–V_{gate} curves of the SWCNT device on the cover glass are shown in Fig. 3, which showed n-type FET properties. When the potential was applied from the back of the device, I_{ds}–V_{gate} curves of the device showed no FET properties because 0.12–0.15-mm-thick glass was a sufficiently high-dielectric material that it intercepted the electrical flux line from the back gate. The I_{ds} increased with increasing the gate potential, which suggests that the SWCNT device in Fig. 1f shows n-type FET. In Fig. 3, I_{ds} at –2 V of the gate potential did not reduce to zero. That phenomenon might occur because of existence of conductive SWCNTs between the source and drain electrodes. Controlling the number of SWCNTs and selective immobilization of semi-conductive SWCNTs are left as objectives of future studies.

A CNT device on the silicon oxide layer is known to show p-type FET as a result of the electron withdrawal of the CNT by adsorption of oxygen gas [14]. Effects of molecule binding to the CNT surface have been investigated by many research groups. Reportedly, the APS molecule changes the electric properties of the CNT-FET device from p-type to n-type [15]. In this study, a lone pair of amino group of APS molecules can become an electron donor and the CNTs will be doped negatively to overcome the effect of the oxygen molecules. These results suggest that a p-type CNT-FET device was fabricated on the glass substrate before fabrication of the insulator by APS on the CNT device (Fig. 1e).
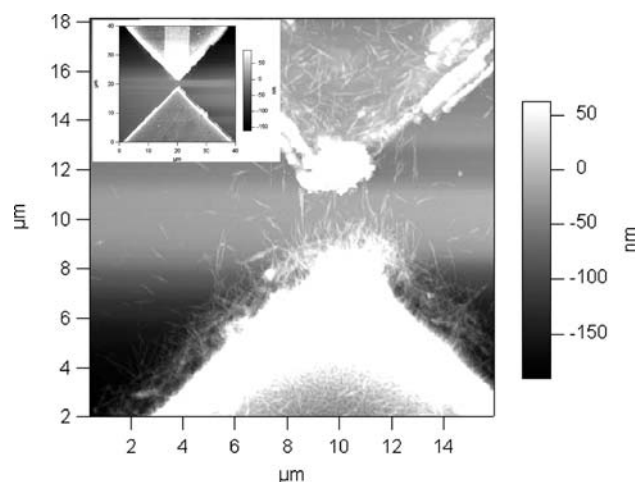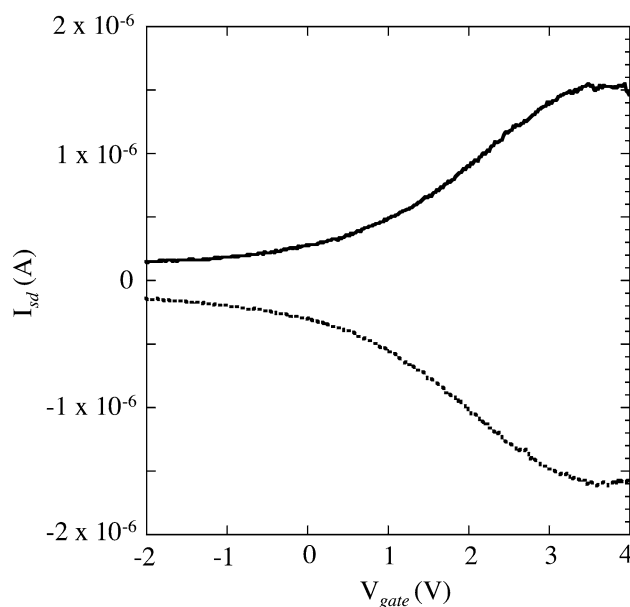

Fig. 2 Surface topography of the electrode planned site on the cover glass after immobilization of CNT on it. The inset shows a comprehensive image of the surface

Fig. 3 A typical I_{ds}–V_{gate} curve of the rope CNT elements built on a cover glass substrate. The potential of 0.1 V (solid line) and -0.1 V (dotted line) was applied to the source electrode; 0 V was applied to the drain electrode.

pH detection using the CNT-FET device on the glass substrate

We also investigated the effect of pH on the SWCNT-FET device because aggregation of the aqueous SWCNT dispersion is controllable by changing the pH. For this experiment, we used a SWCNT-FET device without the insulator layer, as shown in Fig. 1e.

We investigated the properties of the CNT-FET device shown in Fig. 1e in buffer using the Ag/AgCl electrode as the gate so that the buffer on CNT acts as a *liquid gate*. The I-$V_{liquid\ gate}$ curves are shown in the inset of Fig. 4. The I_{ds} increased with decreasing the gate potential, which suggests that the SWCNT device in Fig. 1e shows *p*-type FET and that positive hole in SWCNT is major carrier. This result is compatible with those described in section ''Fabrication of CNT-FET on the cover glass substrate''. The droplets of various pH buffers were dropped on the SWCNT-FET on a glass substrate. The current increased or decreased after dropping various pH solutions with high reproducibility (Fig. 4). Because a proton is an electron acceptor, the proton was able to bind to the SWCNT surface. It accepted an electron from the π-orbital of the SWCNT. Acceptance of the electron from the SWCNT induces generation of a positive hole in the SWCNT; decreasing the pH would increase generation of electron hole in *p*-type SWCNT and contribute to increasing the I_{ds}. The response of the current was higher in an acidic condition, pH 3–5, than in almost

neutral pH of pH 6–8. These phenomena seem to be attributable to the carboxylic acid groups that were introduced on the surface of the SWCNT by a CNT washing processes. At neutral pH, the carboxylic acid is dissociated and positively polarized carbon atom of the carbonyl group is neutralized by resonance effects from oxygen anion. However, the carboxylic group is protonated at pH 3 and the positively polarized carbon atom is not stabilized by the resonance. The positively polarized carbon atom might withdraw an electron from SWCNT, which might subsequently induce generation of positive hole in SWCNT. However, detailed elucidation of the principle of pH sensing using the device remains as a subject for future study.

Measurement of pH change on a local area on a subcellular compartment, a cell, or a cell tissue is crucial to clarify the mechanism of the reaction or signal transfer from a biological point of view. By cultivating the cell or immobilizing a liposome with a membrane protein on the CNT device, we could investigate the local pH change on the cell tissue using the CNT-FET and an optical microscope simultaneously because the size of the cell is greater than micrometer scale, whereas the CNT device size is submicrometer scale. Because glass substrates are commonly used for cultivating cells, CNT-FET on a glass substrate might provide advantages over the use of MOS-FET or commercial pH meters for detection of pH change.

These results suggest that the changing environments around the SWCNT were detected using the SWCNT-FET on cover glass; it might detect various kinds of biomolecules by modification of carboxylic acid of SWCNT or adsorption of biomolecules such as CNT-FET on a silicon oxide substrate. Because the SWCNT on the device has many carboxylic acid groups, efficiency of the immobilization of a biomolecule might be higher than other fabrication methods that contribute to the sensitivity of the sensor.

These results also indicated that, because the sol-gel layer of APS was formed easily on various substrates, SWCNT-FET can be built not only on a cover glass but also on plastic, ceramics, silicon oxide, and so on. The SWCNT-FET device presents the opportunity for combined construction of other apparatus such as optical microscopes, which are sometimes used as a TIRF system for observing a single-molecule level interaction by this device.
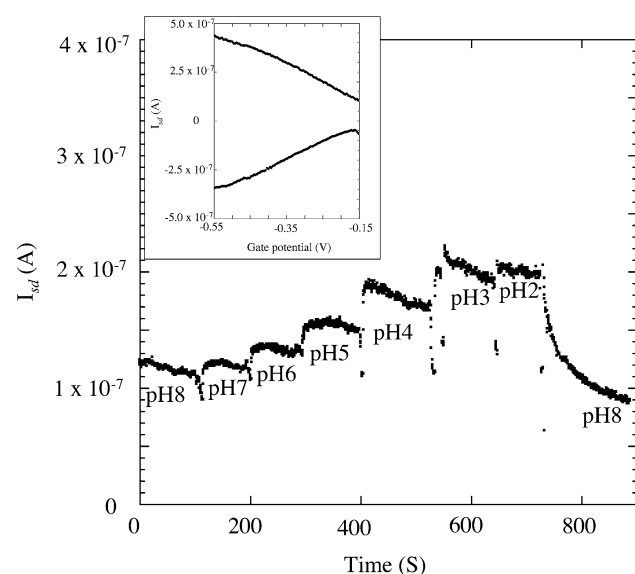

Fig. 4 Effect of pH on current through the source and drain electrode. The CNT-FET was immobilized on a glass substrate. The potential between the source and drain electrodes is 0.1 V. The inset shows I–$V_{liquid\ gate}$ curves of the device

Conclusion

Using the sol-gel layer of APS for immobilization of SWCNT between the source and drain electrode, a SWCNT-FET device can be fabricated on a commercial

cover glass. The CNT-FET device shows *p*-type FET properties. This device, which can detect pH around the CNT, might be applicable for biosensors through modification of the CNT surface.

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