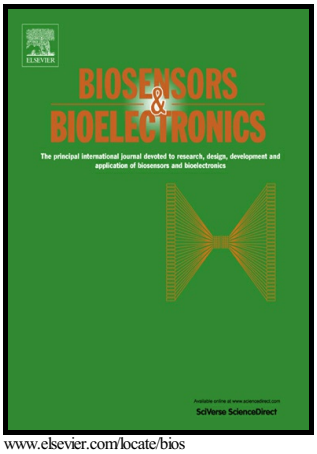

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Detection of early stage prostate cancer by using a simple carbon nanotube@paper biosensor

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Abstract

This study is an investigation for an inexpensive, simple and sensitive biosensor to detect prostate cancer using bioactivated-multi wall carbon nanotubes (MWCNTs, diameter of 20 nm, length of 5 μm) and a micro-pore filter paper (pore size of 0.45 μm). For the immunoassay of prostate specific antigen (PSA), which is a biomarker of prostate cancer, MWCNTs were activated with PSA antibody (monoclonal antibody of the prostate specific antigen) by using N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysulfosuccinimide sodium salt (NHSS). The activated MWCNTs were deposited on the micro-pore filter paper to use as a biosensor. The prepared biosensor can assay from 0 to 500 ng/mL of PSA level within 2 h with the detection limit of 1.18 ng/mL by the measurement of resistance change. The resistance change was caused by site selective interaction between PSA and PSA-antigen with an inexpensive bench top digital multimeter (5 1/2 digits). The detection range and sensitivity of the prepared sensor are good enough to diagnose the early stage of prostate cancer (> 4 ng/mL of PSA). This paper-based biosensor is about 20 times cheaper (fabricated biosensor price: 2.4 $) and over 10 times faster than enzyme-linked immunosorbent assay (ELISA), which is a general method for the detection of a specific protein in the modernized hospitals. Furthermore, the maximum detection limit is about 50 times higher than ELISA.

Keywords: MWCNT, Paper, Biosensor, Prostate, Cancer, Easy detection

1. Introduction

Prostate cancer is the most popular cancer and the third leading cause of cancer deaths among men in the United States (Fedewa and Sauer, 2017). About one in seven American male will be diagnosed with prostate cancer during his lifetime. According to a report (Oh et al., 2016), on the other hand, the incidence rate of prostate cancer in other countries like Korea is still much lower than that in the American. In 2013, the incident rate was 37.6 per 100000 in Korea and one of 450 men will be diagnosed with prostate cancer during his lifetime (Oh et al., 2016). But the incidence rate has been steadily increased in Korea recently, ~10% increased from 1999 to 2013 (Oh et al., 2016). The incident rate of prostate cancer seems closely related to the daily living style. Therefore, prostate cancer might be a major cancer in Korea and other underdevelopment countries not far future because their daily living style has been changed to the western life style during last couple of decays. The detection of early stage of prostate cancer is extremely important to ensure high survival rate. The early detection increases the 5-year survival rate nearly 100%, but it drops to 31% for distant stage (DeSantis et al., 2014; Siegel et al., 2016; Fedewa and Sauer, 2017). The popular early detection method of the cancer is related to the quantification of ultra-low level of a special biomarker of the cancer.

A biomarker is an indicator which identifies any biological states or conditions of the living body. Prostate specific antigen (PSA) is a biomarker of prostate cancer. The normal level of PSA in human serum is less than 4 ng/mL. If the concentration of PSA is higher than 20 ng/mL in human serum, the presence of the prostate cancer is expected.(Thompson et al., 2004) Generally, enzyme-linked immunosorbent assay (ELISA) has been utilized as a method for the PSA detection in the modernized hospitals.. However, this conventional method is a time consuming (approximately 1 day) and a uneconomical (approximately 55 $ per test in Korea) procedure. And the detection should be performed in a well-equipped laboratory. Therefore, the realization of a smart biological detection system, which can do promptly assay the PSA at a few nanograms per milliliter level by a simple

and inexpensive method, will be a greatly advanced step to increasing the survival rate of prostate cancer patients.

There have been many reports for the improved performance of the biosensor (Baaske et al., 2014; Wu et al., 2013; Ruecha et al., 2014; Cai et al., 2014; Wang et al., 2014). The most promising one among the reports is carbon nanotubes (CNTs) based biosensor. Since their discovery in 1991 (Iijima, 1991), CNTs have attracted increasing interest for many potential applications because of their special geometry and physicochemical properties (Wang et al., 2003). The unique properties of CNTs, such as high chemical stability, high electrical conductivity, good mechanical strength, high aspect ratio and larger surface, make them extremely suitable for developing biosensors (Luo et al., 2005).

Many researchers have demonstrated CNTs-based biological sensing systems; electrochemical method based on ionic liquid-CNTs modified electrode (Aliakbarinodehi et al., 2016), electrochemiluminescence method based on quantum dots attached CNTs (Liu et al., 2014), and electronic method based on CNT field-effect transistor (Kim et al., 2009). The detection limit of these biosensors was in the range of 1.0 ng/mL to 0.61 pg/mL. Although these biosensors have advantages of the high sensitivity and fast detection time, the applied technologies contain disadvantages such as high cost, complexity and laboratory-oriented experiment. There was a remarkable claims by J. T. Andraka on the use of the CNT as a biosensor (Andraka, 2013) He claimed a new, rapid and inexpensive biosensor based on the CNTs and a filter paper for the detection of biomarker of pancreatic cancer – mesothelin, but his claim seemed unacceptable in scientific society because of the lack of logic in experimental approach. For example, the mesothelin will not be a biomarker of pancreatic cancer, because the level of mesothelin of health person (0.58 (0.15-0.72) nmol/L) and pancreatic cancer patients (0.66 (0.52-0.94) nmol/L) is not far different. (Sharon et al., 2011) The other bright side of the CNT on paper based sensor was unveiled by a recent report of Koo. They showed that sophisticatedly prepared CNTs on cellulose paper sensor can

be a good choice for an electrochemical analysis (Koo et al., 2016).

It is well known that the CNTs are p-type narrow gap semiconductor in the ambient air owing to the charge transfer from CNTs to oxygen, water and other adsorbed chemicals (Jiang et al., 2014). The same charge transfer occurs also at the interface of metal and CNTs due to the lower Fermi level of metal than that of CNTs (Jiang et al., 2014). Therefore, if the biomolecules attached on CNTs can withdraw electrons from CNTs, the increased concentration of hole in CNTs will enhance the electrical conductivity – hole generation. The electrical transport properties of CNTs thick layer or mat, which were deposited on the surface of an insulator substrate, will be related to the contact potential between conducting CNTs also, because the total transport occurs by percolation process in the system.(Li et al., 2007) If each CNTs in the CNTs layer are coated by insulation layer (i.e. biomolecules) without charge transfer between the insulation layer and CNTs, the electrical conductivity of the total system will be decreased by the increased contact potential – potential barrier generation. These two contradictory effects (hole generation and potential barrier generation) on electrical conductivity of the CNTs system are the basic principle of bio-detection. There are already some reports showing that the resistivity of the biomolecules-CNTs hybrid structure can be modulated by the amount of the attached biomolecules (Yu et al., 2006; Yun et al., 2007; Kim et al., 2009). For example, Kim et al. showed that the large gate effect of biomolecules in the CNT-FET depended on the accessibility of PSA to the CNT surface by using 1-pyrenbutanol as a spacer (Kim et al., 2009). M.B. Lerner and coworkers claimed that the source drain current of the bio-functionalized CNT-FET to detect osteopontin (OPN), which is another biomarker of prostate cancer, is depended on the ultralow level of concentration of OPN (Lerner et al., 2012).

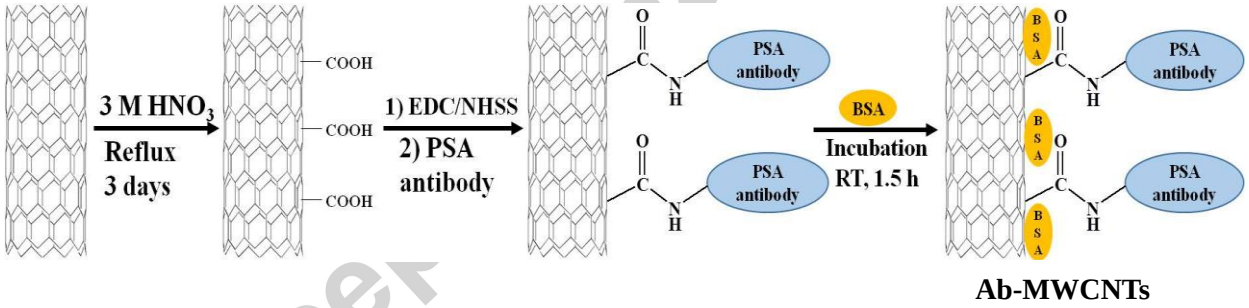
In this work, we report the fabrication and performance of a sensitive, inexpensive and simple biosensor for early detection of prostate cancer, which based on PSA antibody attached multiwall carbon nanotubes (MWCNTs) loaded on micro-pore filter paper. Even though the prepared sensor uses only primary antibody, the detection limit (1.18 ng/mL) of the sensor is noteworthy as

compared to that of the commercially available ELISA kit, 51.0 pg/mL. We believe that this detection method will provide especially useful diagnosis tools for various fatal diseases in undeveloped areas in the world, where the modernized medical systems have not been provided yet.

2. Materials and methods

Chemicals and materials

Nitric acid (64.0~65.0%) was purchased from Duksan science. Phosphate buffered saline (PBS, pH = 7.2) was purchased from Biosesang Inc. 1 M 2-(N-morpholino) ethanesulfonic acid (MES, pH = 5.0) and 0.05% Tween 20 + 1% bovine serum albumin (BSA) in PBS were purchased from Tech & Innovation. N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), N-Hydroxysulfosuccinimide sodium salt (NHSS), monoclonal anti-KLK3 antibody produced in mouse (species reactivity is human, PSA antibody) and prostate specific antigen from human semen (PSA) were purchased from Sigma-Aldrich. Micro-pore filter paper (mixed cellulose ester, diameter 25 mm, pore size 0.45 μm) was purchased from Toyo Roshi Kaisha, Ltd. Multi-wall carbon nanotubes (MWCNTs, diameter 20 nm, length 5 μm) were purchased from Carbon Nano-material Tech. Co. The MWCNTs were produced by chemical vapor deposition (CVD) using iron and molybdenum catalyst.



Scheme 1. Preparation of the activated MWCNTs with PSA antibody.

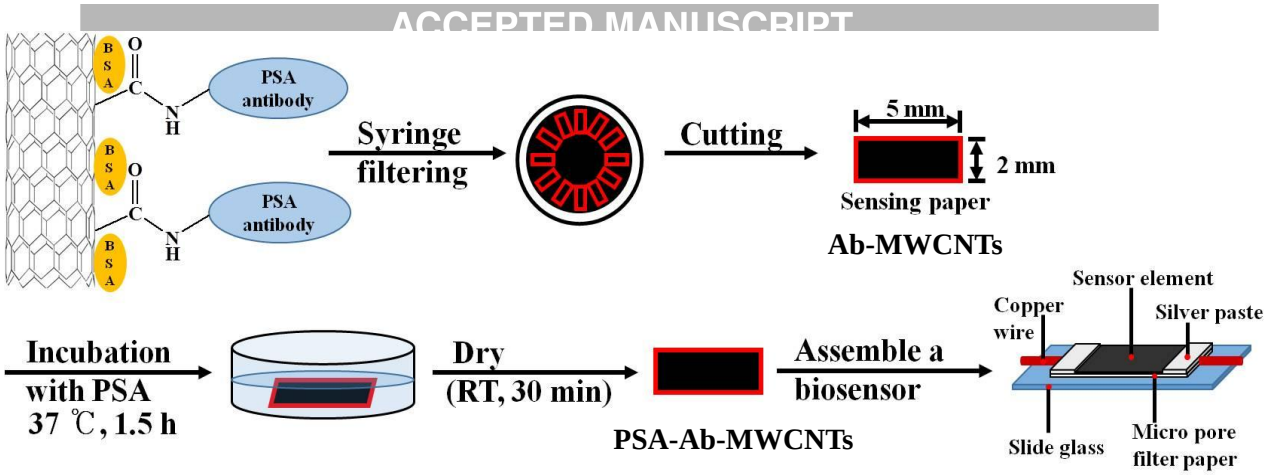
MWCNTs (300 mg) were refluxed in 20 mL of 3.0 M nitric acid at 105 ℃ for 3 days to generate carboxyl group on the surface of MWCNTs (Ren and Guo, 2005). The functionalized MWCNTs were washed and filtered more than 10 times with distilled water until pH was ~7. The washed MWCNTs were dried at 100 ℃ for 24 h in oven. The carboxyl groups on the surface of MWCNTs

were confirmed by a Fourier transform infrared spectrometer (FT-IR, JASCO FT/IR-4100). The first step in Scheme 1 is this carboxylation process.

The carboxyl groups on the surface of MWCNTs could accommodate PSA antibody by following reported steps (Yu et al., 2006). The carboxylated MWCNTs (1.3 mg) were dispersed in 0.1 M MES buffer 4 mL. The buffer solution contains 0.4 mmol EDC and 0.1 mmol NHSS. This dispersion solution was sonicated in a sonicator (Jeio Tech, UC-02) for 1 min and mixed with a vortex mixer (Scientific Industries, Inc.) at room temperature for 15 min. The resulting mixture was centrifuged (Hanil Science Industrial Co. Ltd., HA-1000-3) with 3000 rpm for 10 min, and the supernatant was discarded. At least 5 times washing with PBS were performed to remove excessive EDC and NHSS from the precipitation. The PSA antibody, 0.5 mL (0.01 mg/mL), was added to the mixture and stirred in a vial for overnight at room temperature to attach PSA on the surface of functionalized MWCNTs. This procedure is shown at the second step in Scheme 1.

The important stage of the preparing the MWCNTs based biosensor is prevention of unwanted interaction between the surface of MWCNTs and chemicals (including PSA). A mixed solution of 0.05% Tween 20 in 1% BSA (100 μL) was added into the above PSA antibody added mixture (2 mL) to avoid a random attachment of chemicals on the surface of MWCNTs. The BSA inhibits all non-specific bindings with the sensing element (Niu et al., 2011; Liu et al., 2012) and Tween 20 cleans the binding site of PSA antibody (Yu et al., 2006). The mixture was incubated at room temperature for 1.5 h. The resulting mixture was centrifuged at 3000 rpm for 5 min, and supernatant was removed. The precipitate was washed and centrifuged with PBS buffer 5 times to remove free PSA antibody and BSA. This procedure is shown at the third step in Scheme 1. The sample prepared by this stage was named as Ab-MWCNTs, which stands for PSA antibody attached on MWCNTs.

The Ab-MWCNTs were deposited on a micro-pore filter paper by the syringe filtering. This stage is the first step in Scheme 2. To keep the activity of the PSA antibody, the Ab-MWCNTs deposited on the micro-pore filter paper should not be dried until the completion of the PSA sensing.



Scheme 2. Preparation of sensing element and PSA detection procedure.

The Ab-MWCNTs deposited filter paper was cut into sensor elements in 5 × 2 mm^2 strip. The filter paper should be cut from the center to the circumference as shown in the second step of Scheme 2. By this method, a dozen of biosensor elements could be prepared from one filter paper. This cutting method was very important to keep the constant resistance for all sensor elements, which prepared from the one same filter paper. A drop (100 μL) of PSA contained PBS solution (0 ~ 1000 ng/mL) was loaded on top of the sensor element, which placed in a plastic cell with a seal cap. Then the sensor element was incubated at 37 ℃ for 1.5 h and dried in the air at room temperature for more than 30 min. The sample prepared by this stage was named as PSA-Ab-MWCNTs, which stands for PSA attached on Ab-MWCNTs. The resistance of the incubated sensor was measured by using a digital multimeter (Hewlett-Packard Co. multimeter 3478A or Agilant 34401) at the ambient condition. Silver paste (Dottite) was used to keep the electrical contacts between the sensor element and electric leads (copper wires). See the supplementary information (Fig. S1) for the optical image of the sensor element. The morphological information of materials was observed with an atomic force microscope (AFM, Park scientific instruments) and a scanning electron microscope (SEM, HITACHI S-2400).

3. Results and discussion

1) Bio-activated MWCNT

Fig. 1 shows IR spectra of (a) MWCNTs, (b) carboxylated MWCNTs for 3 days in nitric acid and (c) Ab-MWCNTs. It is clear that the peak position corresponding to the carbon-carbon double bond of MWCNTs is 1630 cm^{-1} in Fig. 1(a). And the peak at 1715 cm^{-1} corresponds to the C=O bond of the carboxyl group, which was generated by the nitric acid treatment of MWCNTs in Fig. 1(b). The carboxyl group at the surface of MWCNTs can react with the amine group of L-lysine unit in PSA antibody as well known (Yu et al., 2006). This is a simple substitution reaction to form the amide group. Before the reaction, the carboxylated MWCNTs were treated with EDC/NHSS to induce a substitution reaction between the hydroxyl group in carboxyl group and the antibody (Yu et al., 2006). The spectrum of Ab-MWCNTs (Fig.1 (c)) shows the presence of the amide group; N-H band near 1560 cm^{-1} and C-N stretching band near 1164 cm^{-1}. See supplementary information (Fig. S2) for the magnified IR spectrum near the assigned band region.

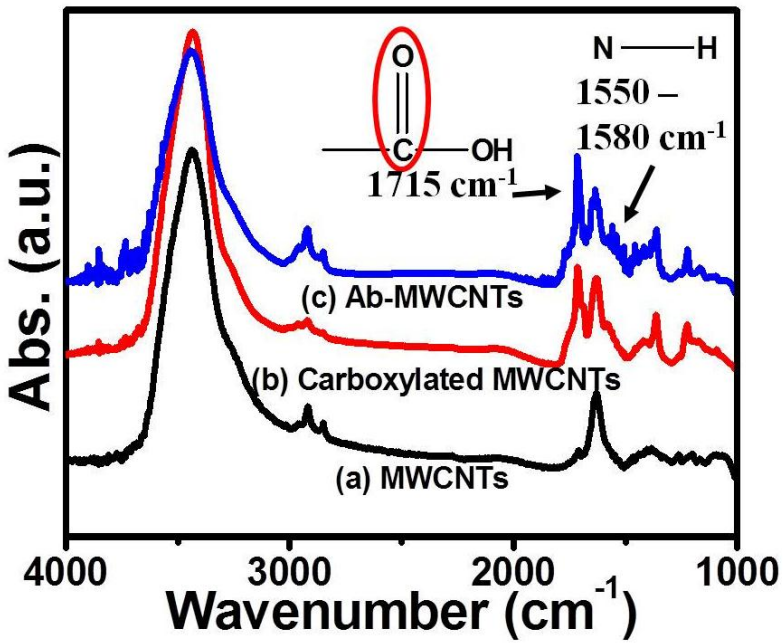


Fig. 1. FT-IR spectra of (a) MWCNTs, (b) carboxylated MWCNTs and (c) Ab-MWCNTs.

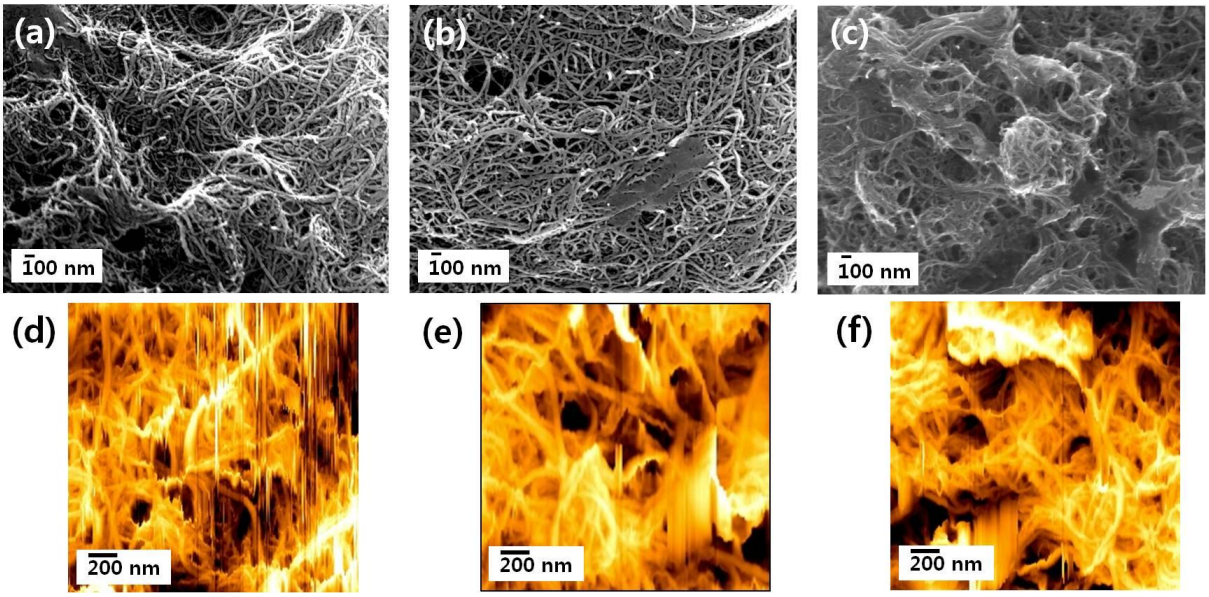
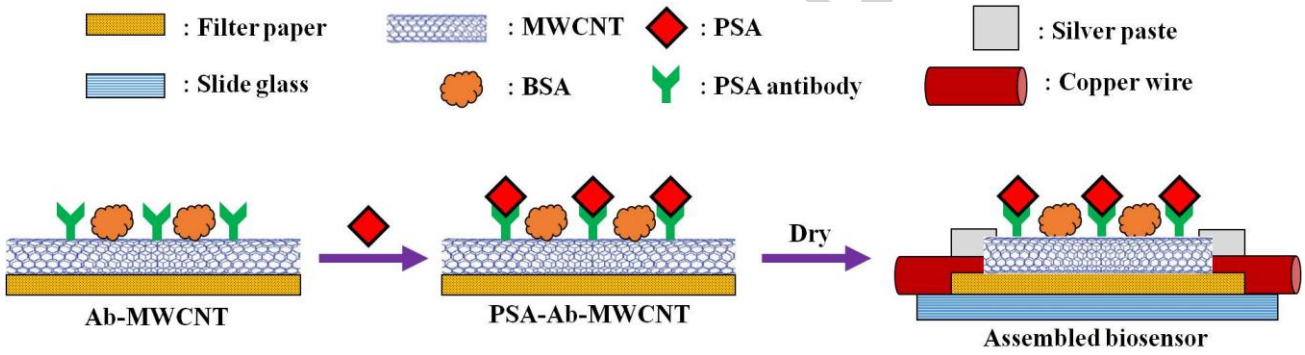


Fig. 2. SEM and AFM images of MWCNTs based sensor element, (a) and (d) are SEM and AFM images of carboxylated MWCNTs, (b) and (e) are SEM and AFM images of Ab-MWCNTs, (c) and (f) are SEM and AFM images of PSA-Ab-MWCNTs.

Fig. 2 shows SEM and AFM images of the MWCNTs based elements. The samples were deposited on a filter paper without the BSA blocking process and then dried at ambient condition for more than 2 h to obtain these images. Fig. 2(a) and (d) show SEM and AFM images of carboxylated MWCNTs. There was no observable damage on the surface of carboxylated MWCNTs. Each MWCNT could be distinguished from both images. The average diameter of the carboxylated MWCNTs was ~20 nm. The images in Fig. 2(b) and (e) are SEM and AFM images of the Ab-MWCNTs layer, which was prepared by the bio-activation under the buffer solution and filtered by micro filter paper to take both images. The MWCNTs in the images are coated by a layer of PSA antibody and BSA (diameter is ~ 4nm). The observed average diameter of the Ab-MWCNTs (~25 nm) was less than expected diameter (~28 nm), because of the protein shrinking in the dry condition. Therefore, morphology of ab-MWCNTs, Fig.2 (b), looks to be close to the carboxylated MWCNTs (Fig.2 (a)). We believe that the cluster, appeared in the middle of SEM image (Fig. 2(b)), may be the result of the localization of excess concentration of antibody.

Fig. 2(c) and (f) are SEM and AFM images of PSA-Ab-MWCNTs. The sensor element was

exposed to the extremely high level of PSA (1000 ng/mL) and dried. In the image Fig. 2 (c), a dense spider web-like surface was appeared distinctively over the film. The spider web-like layers are not clearly observed in AFM images due to the softness of the proteins. This implies that the MWCNTs are covered with the proteins (PSA antibody and PSA). The attachment of the PSA on the Ab-MWCNTs will increase the electrical resistance by the increment of the thickness of protein layer between MWCNTs. The thickness and dielectric constant of the layer is the most important factor in the percolation process, which is the main possible electrical conduction mechanism of the PSA-Ab-MWCNTs, (Li et al., 2007) as discussed in later section. The compactness of the deposit film observed by SEM was in order of carboxylated MWCNTs ≥ Ab-MWCNTs > PSA-Ab-MWCNTs. The estimated root mean square (RMS) roughness from the AFM image of the film was not far different with the microscopic observation; 38, 32 and 61 nm for carboxylated MWCNT, Ab-MWCNT and PSA-Ab-MWCNTs, respectively.



Scheme 3. Site specific detection process of the PSA.

2) PSA detection process

A drop (100 μL) of PSA contained PBS solution (0 ~ 1000 ng/mL) was loaded on the sensing paper (Ab-MWCNTs) in a plastic cell, which must be fit to wet the whole area of the sensing paper. The PSA exposed sensor (PSA-Ab-MWCNTs) should not be dried until the completion of the site-specific reaction between PSA antibody and PSA. Scheme 3 shows the site-specific reaction and following steps to measure the change of electrical resistance in the sensor. The resistance of the sensor related to the wetness of the sensing paper – the resistance decreases during drying process.

In addition to the wetness (or humidity) problem, any other unwanted interaction effects between sensor and impurities could cause a variation of the resistance of sensor. To minimize all the side effects, therefore, the measured resistance of each biosensor was compared to the base resistance of the biosensor. The base resistance is the resistance of a biosensor which incubated Ab-MWCNTs in PBS buffer (0 ng/mL of PSA) at 37 ℃ for 1.5 h and dried for 30 min at ambient air. The sensitivity of the sensor could be maximized by the measurement of the relative resistance of sensor. The measured resistance of each sensor should be corrected with the actual dimensions of the sensing element. But the thickness of the sensing element was not considered for the correction of the resistance, because all the sensing elements were prepared by the same filter paper and the measured resistances were compared with the base resistance. Additionally, the estimation of the film thickness was impossible due to the irregular shape of the loaded MWCNTs layer on the porous filter paper. Therefore, the most important factor in this sensing method is the uniformity of the MWCNT layer, which deposited on a micro-pore filter paper, to keep a constant base resistance of each sensing element.

The uniformity of the MWCNT layer could be achieved with 200 mL of the MWCNT solution, 6.5 μg/mL. The estimated thickness of the deposited MWCNT layer was about 4.5 μm. Also, it is very important to cut the sensing element from the center with a circular shape for keeping the uniform resistance as shown in the Scheme 2. But still we feel the lack of uniformity in the elements. The uniformity is related to detection limit of the biosensor as discussed in later section.

3) Possible detection mechanism

I-V curves of the MWCNTs, carboxylated MWCNTs, Ab-MWCNTs and PSA-Ab-MWCNTs loaded filter papers are shown in supplementary information (Fig. S3). All the layer of MWCNTs obeys the Ohm's law at high current range (from -1.4 to +1.4 mA) as well as low current range (from -1.0 to +1.0 μA). The electrical resistance of each MWCNTs layer could be estimated from the slope

of the plot and the change of relative resistance depicted in supplementary information (Fig. S4). The electrical resistance was increased to 500% by carboxylation of the MWCNTs. The charge transport in MWCNTs happens through the sp^2 pi-band of carbon. The decrement of the sp^2 character by the formation of carboxyl group on the surface layer of MWCNTs will mainly cause the increment of resistance. The electrical resistance of Ab-MWCNTs (PSA antibody attached on the carbonyl group and BSA blocked the surface of MWCNTs) layer was 1.6 times larger than that of the carboxylated MWCNTs. The charge transport between MWCNT filaments in MWCNTs layer will be happened by the percolation process through the crossed areas between MWCNTs.(Li et al., 2007) The attached proteins are electrically insulator and will act as a potential barrier of the percolation process between MWCNTs. Therefore, the increased resistance after the attachment of the proteins (PSA-antibody and BSA) on the carboxylated MWCNTs is originated by the increased insulating thickness between MWCNTs. By the same reason, the additional attachment of PSA to Ab-MWCNTs will result the resistance increment: the resistance of PSA-Ab-MWCNTs (PSA, 1000 ng/mL) was increased 150% higher than that of the Ab-MWCNTs. The gradually increased resistance of CNTs along with the surface modification was displayed in supplementary information (Fig. S4).

If the attached biomolecules on the surface of CNTs can withdraw electrons from the CNTs, the conductivity of the CNTs will be increased (increased off-current) and the thresholds voltage (V_{TH}) of a field effect transistor (FET - assembled with biomolecules-CNTs on electrodes) will be shifted to a more negative voltage due to the increased p-character, vice versa (Hecht et al., 2006; Allen et al., 2007). On the contrary to the charge transfer model, the increase of contact potential between the conductive CNT filaments by the adsorption of biomolecules on the surface of the CNTs will cause the decrease of conductivity without V_{TH} change of the CNTs based FET (Hecht et al., 2006; Allen et al., 2007). Therefore, the amount of target biomolecules, which can be attached on the modified CNTs, will determine the resistivity change of biomolecules-CNTs and the V_{TH} shift of the FET.

However, we could not observe the shift of V_{TH} in our prototypic CNT-FET, which assembled between Au microelectrodes on SiO_2/Si wafer by using Ab-MWCNT, and could observe the decrease of the off-current of the FET by the increased PSA dose level.(not shown here) This indicates the concentration of charge carrier is constant due to the prohibited charge transfer between the CNTs and PSA, and the contact potential between CNTs is increased due to the attachment of PSA at the active site of the CNTs.

Therefore, the possible sensing mechanism is only the site-specific reaction between the attached PSA antibody and PSA in the sensor and any other random interactions between CNTs surface were protected by the BSA blocking (Yu et al., 2006; Kim et al., 2009; Niu et al., 2011; Liu et al., 2012). The site-specific reaction does not allow the direct charge transfer between MWCNTs frame and PSA. And the reaction increases the distance between MWCNTs only. The most important factor of the electrical conduction in MWCNTs-epoxy composite is tunneling resistance between MWCNT filaments.(Li et al., 2013) The tunneling resistance depends on the thickness and dielectric constant (***K***) of the insulator layer between crossed MWCNTs. The most commonly used average value of protein is, K = 4, (Li et al., 2013) very close to that of epoxy, K =3.98. (Li et al., 2007) Therefore, the epoxy coated MWCNTs seem a good model to understand the detection mechanism of our sensor. The tunneling resistance was increased up to order of 10 by the 1 nm thick epoxy layer in the model composite and the resistance of MWCNTs was increased less than 1 order by the coating of PSA, PSA antibody and BSA. This suggests the thickness of the coated protein is very thin in dry condition as discussed previous section and a small change of the insulator thickness causes a large resistance change in the MWCNTs system. Therefore, the increased the resistance in PSA-Ab-MWCNTs will be mainly caused by the adsorption of PSA on the Ab-MWCNTs, which increases the insulating distance between the crossed MWNCTs. The excellent hydrocarbon detection ability of metal attached-CNT based FET could be explained by the same token (Lee and Kim, 2016).

4) Reliability of the sensor

The relative electrical resistance (R/R_o), where R is the resistance exposed to PSA and R_o is the resistance exposed to PBS buffer solution (zero level PSA), of the sensing elements (Ab-MWCNTs) by the reaction with PSA is shown in Fig. 3. We made twelve sensing elements from a batch and checked the relative resistance at twelve levels of PSA (0 ~ 1000 ng/mL) to check the uniformity and linearity at the same time as shown in Figure 3. This was a good possible way to check the performance of the sensors, because the prepared sensor should not be dried until PSA detection process to keep the bioactivity. Fig. 3(a) shows that the change of the relative resistance depends almost linearly on the concentration of PSA in the range of 0 ~ 500 ng/mL. A test of this linearity could be used as an internal standard for a detection to confirm the detection ability of the same batch sensing elements under different detection environments. We achieved the linearity of executed experiments for last 18 month was about 30%. The data points in Fig. 3 are averaged values of the 10 valid experiments. The linear range covers much wider concentration than other PSA detection methods. The linear range for CNT based FET sensors is 0-100 ng/mL (Kim et al., 2009), and that for ELISA is 0 - 10 ng/mL. The maximum detection range of our sensor is 50 times higher than ELISA. The sensing range might be tuned by the degree of carboxylation in the MWCNTs and the thickness of deposited active layer on filter paper.

Fig. 3(b) shows the detectability of the sensor in the low PSA concentration range (0 ~ 10 ng/mL). In the plot, the measured relative resistance shows very good linearity for the concentration of PSA. This means the sensor can detect the low concentration of PSA to find the early stage of prostate cancer. If PSA level is higher than 4 ng/mL, the prostate cancer can be diagnosed as the early stage. The detection limit, which was estimated by 2 times of standard deviation from the average signal of our eleven biosensors exposed to PBS buffer solution, was 1.18 ng/mL. This detection limit is much higher than that of ELISA (51 pg/mL) or some advanced type of CNT based FET (0.61 pg/mL), but

it could be further improved by the optimization of CNTs functionalization, deposition method on the filter paper and others in the near future.

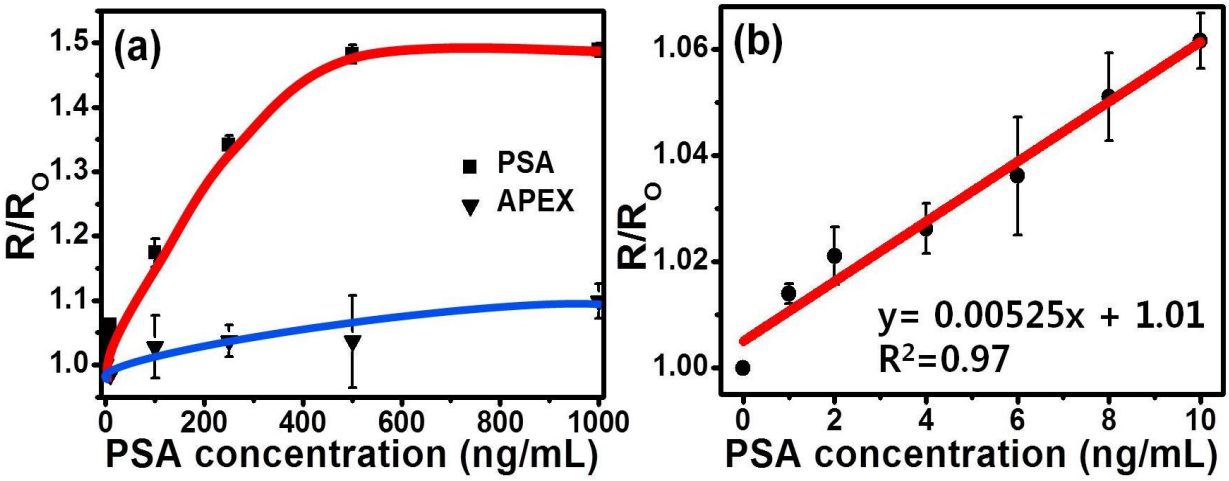


Fig. 3. The relative resistances of the biosensors are plotted in the (a) wide range (0 ~ 1000 ng/mL) and (b) narrow low-range (0 ~ 10 ng/mL) of PSA and APEX concentration.

The selectivity of sensor to PSA is very important in practical applications for a diagnosis of prostate cancer. The relative resistance did not change when BSA coated MWCNTs exposed to PSA without the PSA active site. This reconfirms that the BSA prevents the random adsorption of PSA on the wall of MWCNTs, as reported by others (Ruecha et al., 2014). The selectivity of the active site of the sensor was tested with DNA-(apurinic or apyrimidinic site) lyase, APEX nuclease. The APEX is a multifunctional DNA repair enzyme in human body. The molar mass of APEX (36 kD) is not far different from that of PSA (34 kD). According to a report, the size of a protein can be estimated from the molar mass (Erickson, 2009), the estimated size of APEX (4.4 nm) and PSA (4.2 nm) is comparable. Therefore APEX may not have any additional obstacles to approach the active sites of the BSA coated MWCNTs by a simple diffusion process from the bulk solution. But the sensor only shows the change of baseline noise in the range of 0 ~ 1000 ng/mL of APEX. Therefore, the selectivity of the sensor is originated from the site-specific reaction, in which the active site for PSA (Ab-PSA) interacts only with PSA. In addition to the selectivity, other unwanted random adsorption effects by chemicals in buffer solution could be removed by the comparison of resistances of sensing

element exposed to PSA and the buffer solution:(R/R_o). However, the selectivity to the other type of clinical markers should be considered in actual application.

We have showed the realization of an inexpensive, simple and sensitive biosensor: PSA antibody activated MWCNTs deposited on a micro-pore filter paper. The sensor can assay PSA in a wide range (0 ~ 500 ng/mL) with high sensitivity (detection limit of 1.18 ng/mL) within 2 h by the measurement of electrical resistance (5 1/2 digits) only. The performance of the sensor is good to diagnose the early stage of prostate cancer (> 4 ng/mL of PSA). This paper-based biosensor is much cheaper and faster than ELISA. Furthermore, the sensor could be modified with a proper antibody to assay the specific biomarkers of any other diseases.

4. Conclusion

We have successfully demonstrated a fabrication of sheet-type biosensor based on MWCNTs and micro-pore filter paper for early detection of prostate cancer. The high concentration of PSA increases the electrical resistance of the sensor up to 150%. Maximum detection and the detection limit of the sensor was 500 ng/mL and 1.18 ng/mL, respectively. The paper based biosensor is ~23 times cheaper (fabricated biosensor price: 2.4 $) and 12 times faster than ELISA based method used in a hospital, which is a general method for the detection of a specific protein. Furthermore, the maximum detection level of the paper based biosensor is about 50 times higher than that of ELISA. The detection range and sensitivity of the prepared sensor are sufficient to diagnose the early stage of prostate cancer (> 4 ng/mL of PSA). The paper based sensor could be used to detect various biomolecules as well as PSA by using various bio-specific materials. Therefore, we believe this handy bio-sensing procedure could be a very useful diagnosis tool for fatal diseases in undeveloped areas of whole world, where medical system is poorly equipped, in the not far future.

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Graphical Abstract