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14. ABSTRACT The project aimed to develop a point-of-care diagnostic platform for tuberculosis diagnosis. The sensor detected Mycobacterium tuberculosis (MTB; H37Ra strain) cells and MPT-64 antigen spiked in samples from tongue swabs and sputa. The fabrication protocol using single walled carbon nanotubes were developed for an immuno-resistive sensor to detect target analytes. We achieved and exceeded the original project goals using the developed sensing platform. For sputum samples, the magnetic enrichment of targets was combined with the SWCNT sensors. The detection limits for MTB and MPT64 spiked in sputa were 100 CFU/mL and 1ng/mL, respectively. The detection limits were 10 CFU/mL for MTB and 100 ng/mL of MPT64 in tongue swab samples with the detection time of 30 minutes. The detection limit was comparable to PCR without requiring bacteriological culture, centrifugation, or nucleic acid amplification.					
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Table of Contents

Page

1. Introduction.....	4
2. Keywords.....	4
3. Accomplishments.....	5
4. Impact.....	27
5. Changes/Problems.....	28
6. Products.....	30
7. Participants & Other Collaborating Organizations.....	31
8. Special Reporting Requirements.....	32
9. Appendices.....	33

1. INTRODUCTION:

Subject: This project was to develop a point-of-care platform for tuberculosis diagnosis. The diagnostic sensor successfully identified both *Mycobacterium tuberculosis* (MTB) cells (H37Ra strain) and MPT-64 antigen spiked in human sputum samples and tongue swab samples. This film-type resistive immunosensor detected the target analytes using single walled carbon nanotubes (SWCNTs) patterned on a plastic film. Upon binding of target to the sensor surface, electric resistance was measured to detect the binding of target analytes-MTB cells and MPT-64 spiked in human sputum samples. To enhance the specificity, additional antibodies specific to the TB antigen MPT-64 were raised and tested.

Purpose: The purpose of the project was to develop a more rapid and high performance assay that was also low cost. The assay, faster and cheaper than smear microscopy and PCR, facilitates the development and validation of new TB prevention and treatment methods for military use. The proposed immunosensor will provide a practical solution for the current need for rapid, inexpensive and accurate TB screening in field settings where resources are limited.

Scope: In the project, we aimed to achieve a detection limit of 1,000 CFU/mL (or equivalent detection limit: 125 pg/mL for MPT-64) with a detection time of 20 minutes. To develop a point-of-care assay for tuberculosis diagnosis, the sensor fabrication and functionalization protocols were developed with the optimization of the protocol. The sensor was characterized in benign buffer spiked with MTB and MPT-64. The probe antibodies were raised against MPT-64 to obtain the specificity. For the sensor evaluation, benign sputum samples were spiked with MTB and MPT-64. The changes in the electric resistance through SWCNTs upon binding of the analytes were monitored to detect the targets without culture or PCR amplification. In addition to the sputum samples, we tested the sensor for tongue swab samples. Tongue swabs were tested due to recent research showing it to be a convenient biosample source for TB diagnosis. The detection limit was evaluated by using the developed fabrication and detection protocols.

2. KEYWORDS: MTB, MPT-64, IgY, Carbon nanotubes, Point-Of-Care Diagnosis, Immunoassay.

3. ACCOMPLISHMENTS:

The major goals are described with the specific tasks as below.

Aim 1 (completed: 100%)

Task 1: Design and fabricate SWCNTs-based immunosensors

Task 1A: Fabrication of various dimensions of SWCNT based sensors

Task 1B: Study of SWCNTs doped with various concentrations of PEI

Task 2: Sensor optimization for specificity and sensitivity using pure samples

Task 2A: Preparation and evaluation of antibodies for BCG and MPT-64

Task 2B: Fabrication of antibody immobilized sensors

Task 2C: Evaluation of sensor to sensor variation.

Aims 2a (Task 3) (completed: 100%). Demonstrate the prototype device with pure samples

Task 3A: Preparation of cells and MPT-64

Task 3B: Demonstration of sensor performance for MTB (BCG) in PBS buffer

Task 3C: Demonstration of sensor performance for MPT64 in PBS buffer.

Aims 2b (Task 4) (completed: 100%). Demonstrate the prototype device with simulated sputum samples

Task 1. Design and fabrication of SWCNTs-based immunosensors.

Taks 1A: Fabrication of various dimensions of SWCNT based sensors

The fabrication of the electrodes was done by stamping of the silver ink in the desired shape. As shown in Fig. 1a, the stamping tool was made of a stand supporting the whole apparatus, a handle that allowed for a slow and precise linear motion of the stamp, a graduated scale displaying the displacement of the stamp during motion, the aluminum stamp holder, and a polymer stamp. The stamp (Fig.1b) was made of polydimethylsiloxane (PDMS) cured in a CNC-machined Delrin® mold at room temperature for 3 days. The PDMS stamp coated with silver ink was lowered to transfer the silver ink to fabricate the electrodes.

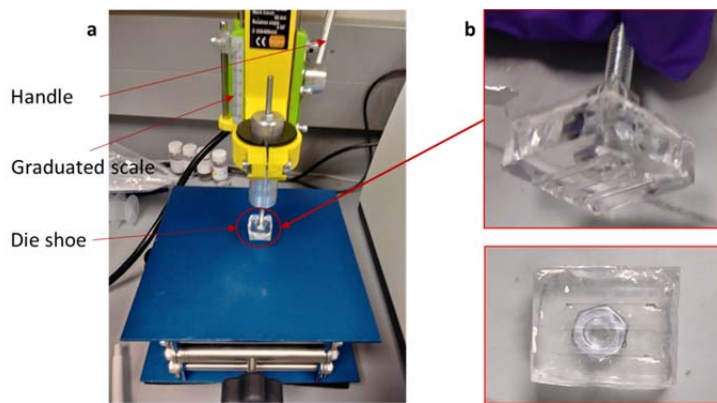


Figure 1. Stamping setup. (a) Stage of the stamping process (b) the stamp made of PDMS. Two setups were constructed, one for Chung's group at University of Washington and a second for Kim's group at Washington State University.

For the printing process, the stamp allowed soft and gentle application of the silver ink from the small ink dish to the polyethylene terephthalate (PET) film. The silver ink was cured at 100°C for 10 min to ensure the total elimination of solvent from the ink. The electrode gap size at the sensing area of the device was varied from 0.5 mm to 3 mm (Fig. 2a, 2b, and 2c). At the end of the electrode, a wide square with a 2mm side ensured adequate contact for amperometric measurement. The fabricated device is shown in Fig. 2d. The gap size of the electrodes was controlled from 3 to 0.5 mm. Two sensors are integrated for multiplexing MPT-64 and MTB in sputum samples.

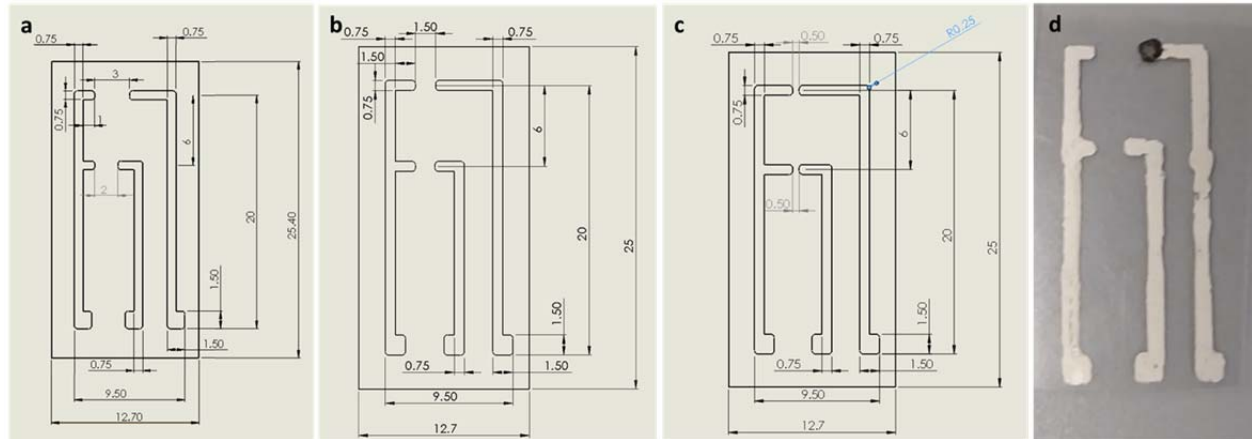
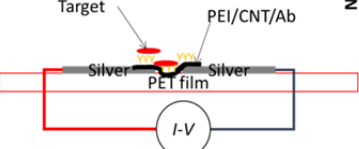
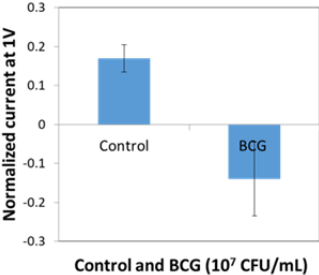
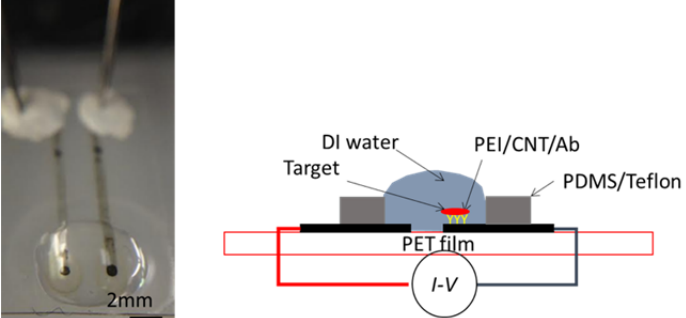
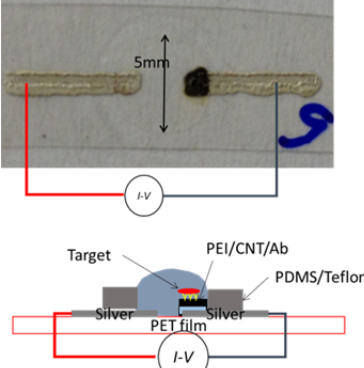
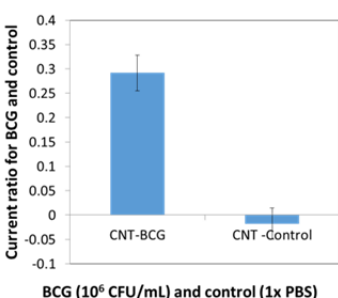
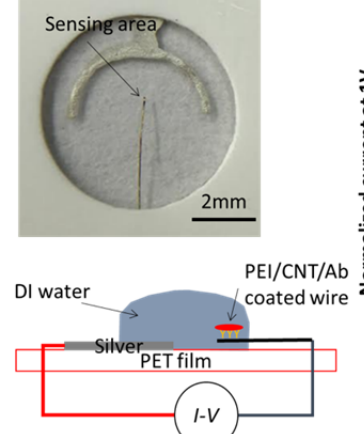
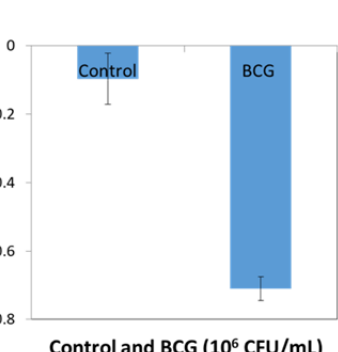
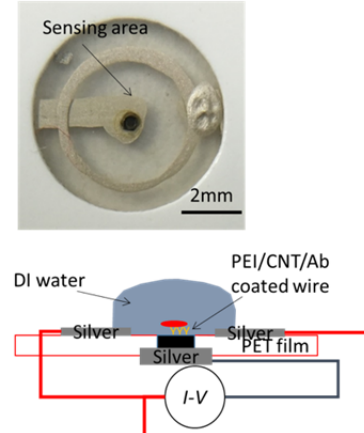
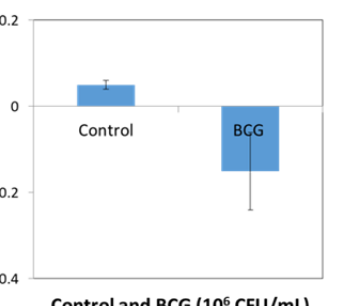


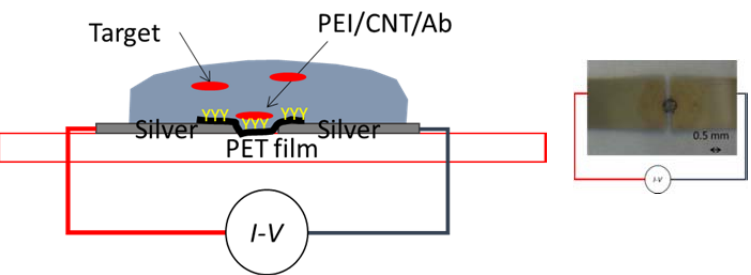
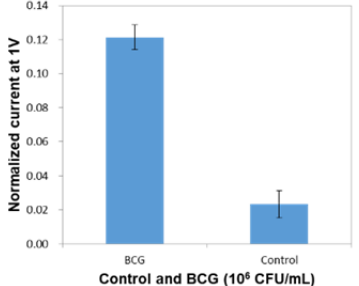
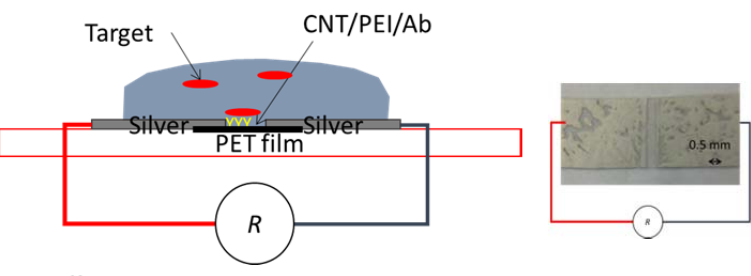
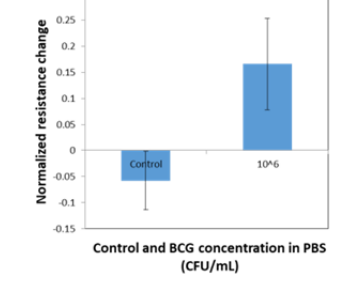
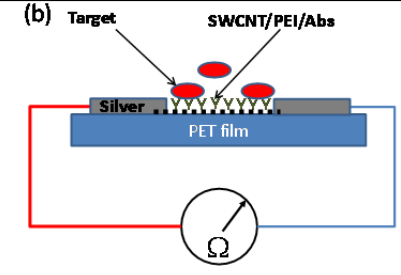
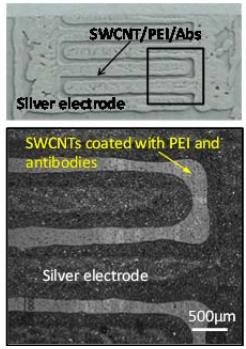
Figure 2. Various electrode configurations. (a) 3 and 2mm gap device. (b) 1.5mm gap device. (c) 0.5mm gap device. (d) Fabricated device

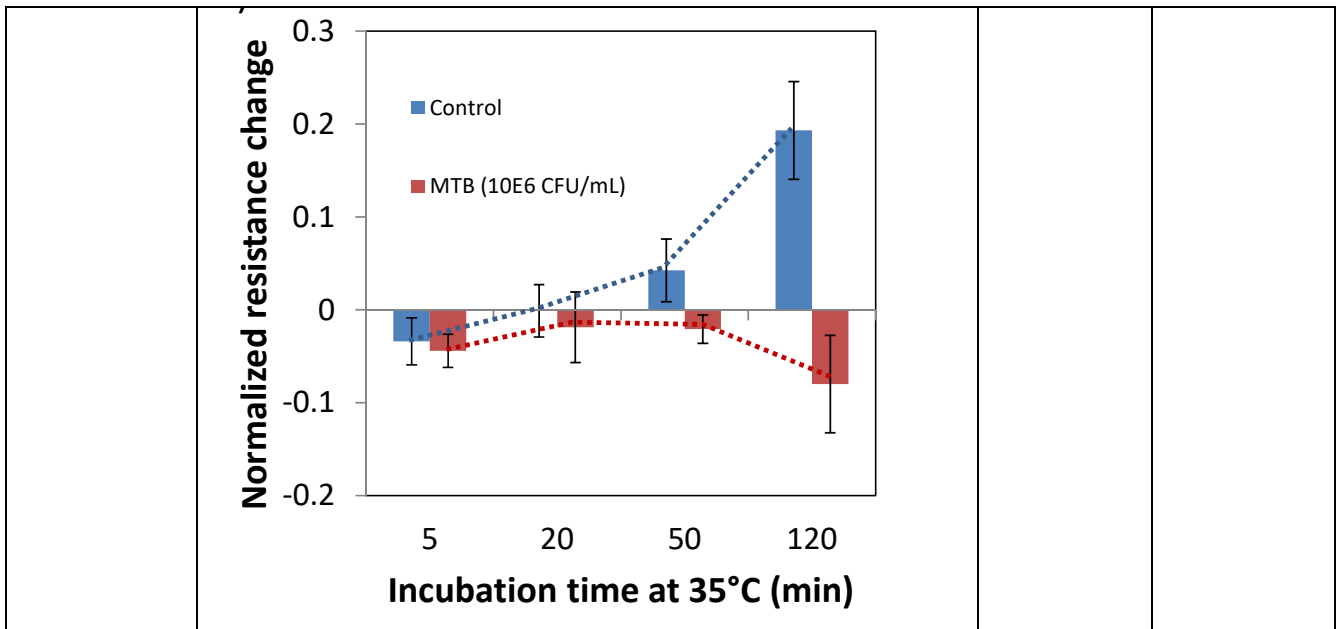
Throughout the project, total 8 devices with different configurations were fabricated and tested. In consideration of the device performance, reliability, and usability, the device shown in number 8 has been chosen for the final tests. The device configurations are summarized with the working principles and the pros & cons in Table 1.

Table 1. Testing results for various configurations of SWCNT sensors

	Sensor configuration, picture and data	Working principle*	Pros & cons
1. Presented sensor (Sept 2017~present)	  	Electrostatic gating effect and Schottky effect; measurement without DI water	Simple fabrication Simple detection protocol

2. Two prong sensor (May 2017~June 2017)		Electrostatic gating effect; Measurement in DI water	Simple fabrication No difference between control and BCG
3. Two electrode sensor (June 2017 ~December 2017)	  Current ratio for BCG and control BCG (10^6 CFU/mL) and control (1x PBS)	Schottky effect and measurement by radial direction of SWCNTs; Measurement in DI water	Simple fabrication Data is not reproducible
4. Wire sensor (January 2018~March 2018)	  Normalized current at 1V Control and BCG (10^6 CFU/mL)	Schottky effect and measurement by radial direction of SWCNTs; Measurement in DI water	Higher sensitivity Fabrication process is not reproducible
5. Micropore sensor (January 2018~March 2018)	  Normalized current at 1V Control and BCG (10^6 CFU/mL)	Schottky effect and measurement by radial direction of SWCNTs; Measurement in DI water	Higher sensitivity Fabrication process is not reliable

6 Larger area sensor	  Control and BCG (10^6 CFU/mL)	Electrostatic gating effect + Schottky effect; Measurement in DI water	Simple fabrication Simple detection protocol. Not reproducible
7. Larger area sensor	  Control and BCG concentration in PBS (CFU/mL)	Electrostatic gating effect ; measurement in 1xPBS buffer.	Simple fabrication Simple detection protocol. Reproducible results
8 Larger area sensor with interdigitated electrodes (Final and optimized sensor configuration)	 	Electrostatic gating effect ; measurement in 1xPBS buffer.	Simple fabrication Simple detection protocol. Reproducible results



Task 1B: Study of SWCNTs doped with various concentrations of PEI

To print SWCNTs, a non-contact printing method using a capillary pen was developed. The noncontact capillary method deposits nanoink through a liquid bridge formed between a capillary pen and the substrate (Fig. 3a). A stylographic pen consists of a capillary nozzle and a rod-shaped ink stopper that assures the nanoink is sealed when the pen is not used. During printing, two geometric parameters require control in order to maintain the capillary bridge integrity: the pen tip height (H) from the substrate and the advancing bridge contact angle (θ_{B_a}) as illustrated in Fig. 3a. When the liquid bridge is established, the ink flow rate depends on the pressure difference between the capillary pen reservoir and the substrate surface. In a static condition, θ_{B_a} is dependent on the substrate surface properties, its temperature, and ink properties. When the pen moves to the right, θ_{B_a} increases, and the recessing angle (θ_{B_r}) decreases. As θ_{B_a} increases, the hydrostatic pressure on the surface increases to reduce the ink flow. For low θ_{B_a} , the pressure difference is maximized resulting in the high ink feed rate.

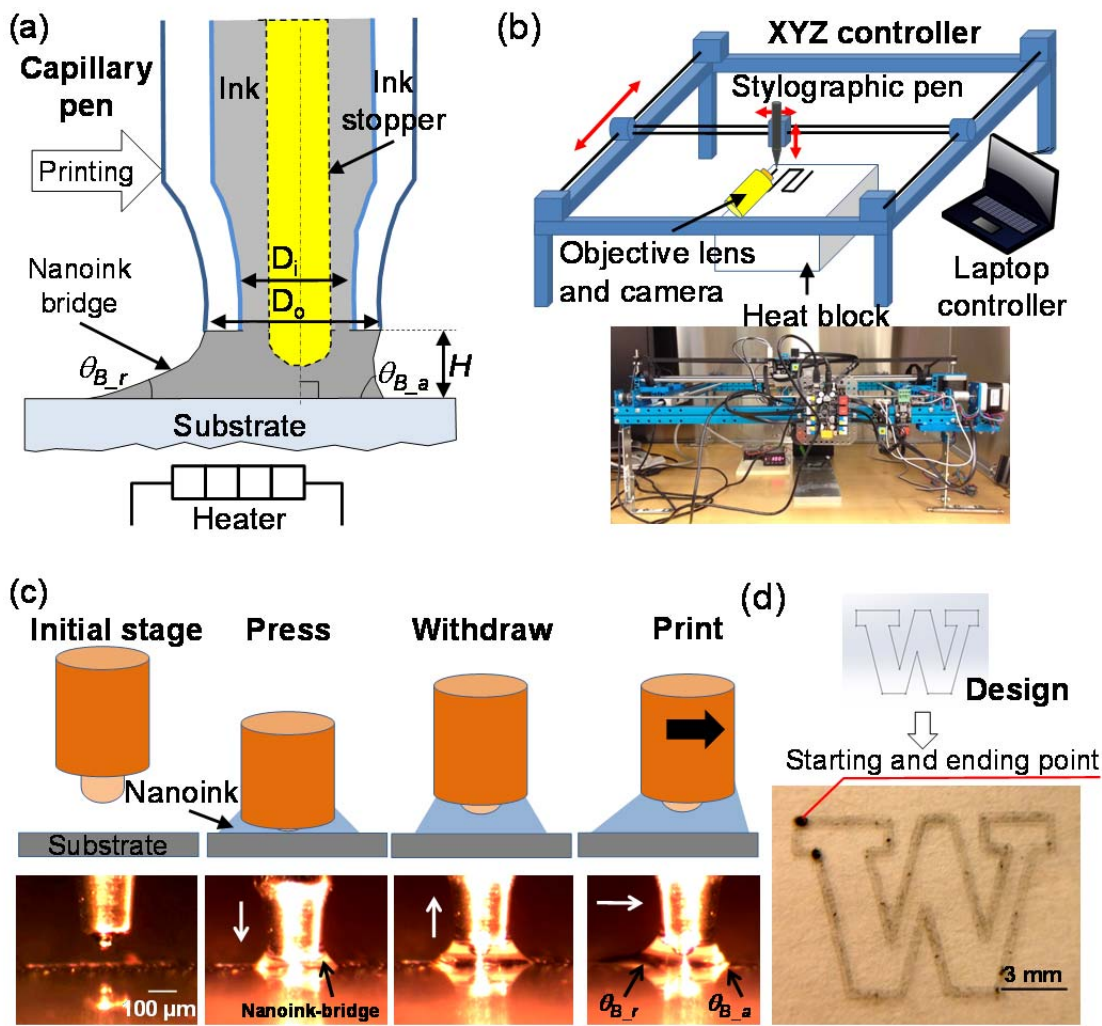


Figure 3. Nanoink bridge induced capillary printing (a) Printing concept (b) Schematic of an xyz plotter installed with a heating stage and a camera system. The top image shows a printing system, and the bottom is a photograph of the setup. (c) Nanoink-bridge induced printing using water ink on a PET film. The ink is released by pressing the stopper. Upon withdrawal by 100 μm , an ink bridge forms. The advancing contact angle increases as the pen moves from left to right. (d) W-pattern printed by SWCNT-ink at 80°C at 1.2 mm/sec. The top image shows the design.

Fig. 3b shows the printing setup consisting of an x-y-z plotter and control module. The printing direction is controlled by two step motors in x and y directions. Manual micropositioning stage sets the Z-coordinate (pen tip height; H) necessary to form a nanoink bridge. A camera with a microscopic objective lens monitors the condition of the liquid bridge for a feedback control. The substrate temperature is controlled via closed-loop by a thermocouple as a sensor for a heating stage.

Fig. 3c illustrates the printing procedure. First, the nib is pressed in the axial direction, and nanoink is released by capillary action. Second, upon the release of the nanoink, the pen is withdrawn from the substrate to $H=100 \mu\text{m}$; a nanoink bridge forms between the pen tip and the substrate. When the pen moves to the right, θ_{B_a} increases and θ_{B_r} decreased. Finally, the pen is retracted to stop the print. Fig. 3d shows a typical example of the printed pattern using the nanoink-bridge printing on a polyethylene terephthalate (PET) film. A PET film is an appropriate substrate

for chemical sensors because it is chemically resistant, transparent and mechanically robust. Note that the details of the printing method are attached as a manuscript (published to Nanotechnology) in the appendix.

For doping of SWCNTs, various amounts of 1% polyethylenimine (PEI) were tested to characterize the PEI doped SWCNTs. PEI, known to effectively interact with CNTs via physisorption on the CNTs sidewalls, was used in this assembly. The high affinity of PEI for SWCNTs led us to use it as an adhesive layer for uniform surface. Two different volumes of PEI solution (0.4 and 0.8 μL) were evaluated based on the electrodes.

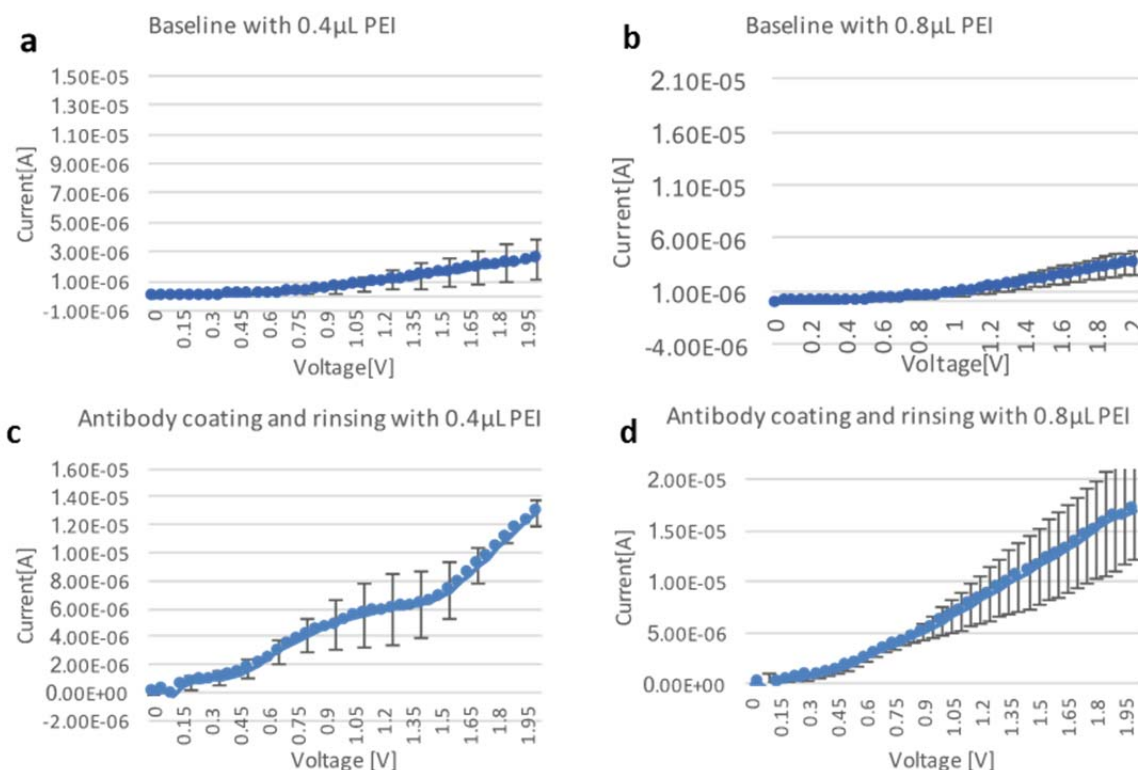


Figure 4. I-V curves for 1% PEI/SWCNTs in SDS coatings. (a) Baseline with 0.4 μL of 1% PEI and (b) 0.8 μL of 1% PEI, (c) I-V curve after antibody coating and rinsing with a 0.4 μL PEI layer and (d) with a 0.8 μL PEI layer.

SWCNTs were dispersed in 1% sodium dodecyl sulfate (SDS in DI water) at a concentration of 1mg/ml using a sonicator at room temperature for 8 hours. Half of the volume of PEI was used for SWCNTs coating. The SWCNTs were also dispersed in DMF at a concentration of 50mg/L using a sonicator at room temperature for 4 hours. The solution was kept stable by storing at -5°C . The low concentration of 50mg/L was used due to the dispersion limit of CNTs in DMF. With the device on a hot plate at 80°C , 0.5 μL of SWCNT in DMF was placed on top of the cured PEI film and dried for 10 minutes. In the process, the carboxyl group in DMF made SWCNTs negatively charged. The amine group in PEI allowed ionic bonding of the SWCNTs with the PEI coated PET film. The SWCNT coating process was repeated 4 times to achieve a firm electrical connection between silver electrodes. The resulting resistance of SWCNT-connected electrodes was $\sim 100 \text{ K}\Omega$. A picoammeter (Keithley, 6487) was used to measure I (current) –V (voltage) curves for characterizing the fabricated devices. The sensor characteristic was measured by testing the initial current of the device. Subsequently, we coated the device with antibodies followed by a rinsing

step to eliminate excess antibodies on the electrode. We, then, carried out another measurement to evaluate how the current was affected by the antibody binding. The only varied parameter at this point of the experiment was the PEI volume in the coating process. Fig. 4 summarizes the I-V measurements with 0.4 and 0.8 μL of 1% PEI. The current reading for both 0.4 and 0.8 μL of 1% PEI was similar as shown in Fig. 4a and b. However, the measurement with the 0.4 μL PEI coating after antibody coating and rinsing showed a smaller error bar around half of the 0.8 μL PEI, meaning that 0.4 μL PEI layer generated more consistent current. This led us to proceed with the smaller volume of PEI coating (0.4 μL PEI).

Task 2: Sensor optimization for specificity and sensitivity using pure samples.

Task 2A. Preparation and evaluation of antibodies for BCG and MPT64

In this task, BCG cells and MPT64 protein were prepared. The previous raised antibodies against BCG were used. The antibodies to the MPT64 protein were newly raised and evaluated.

Antibodies to the MPT64 were raised in two hens and evaluated by ELISA to determine binding to protein, and filter plate EIA to determine reactivity to cells. The purified total IgY fraction was analyzed by ELISA and compared to the pre-immune IgY from the same hen.

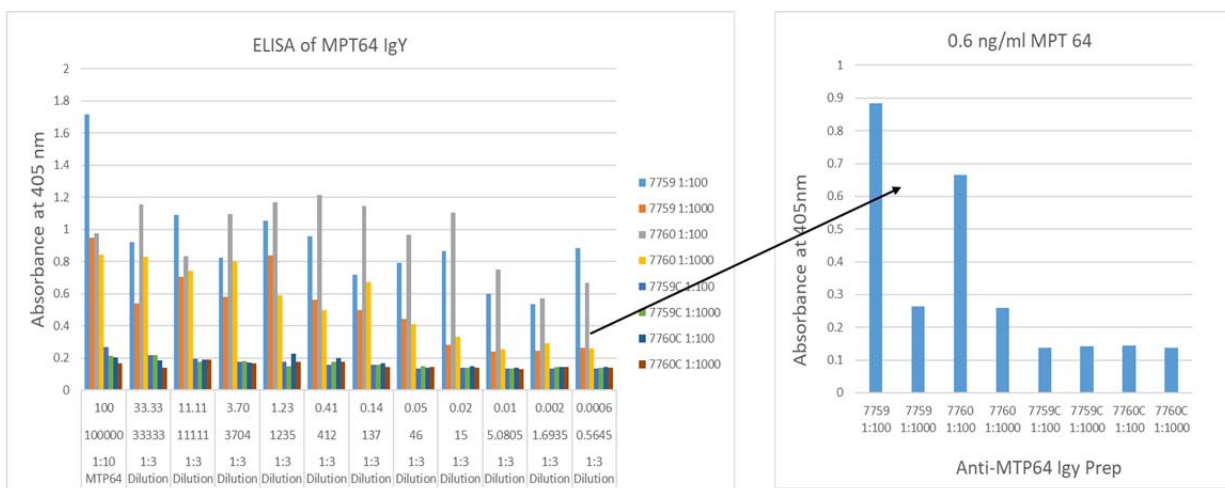


Figure 5. (Left). Analysis of the two IgY antibodies generated to the MPT 64 protein. The antibodies, labelled "7759" and "7760" were run at two dilutions (1:100 and 1:1000), and compared to the pre-injection (control) antibodies from the same hen at the same concentration. MPT 64 protein was coated on the plate surface (1mg/ml stock) by diluting 1:10 in the first well, followed by serial 1:3 dilutions. (Right) Concentrations as low as 0.6ng/mL were detected. A larger volume of antigen in the last column (0.6ng/ml MPT64) resulted in a slight increase in signal over the previous dilution.

An ELISA was first performed to evaluate the reactivity to the purified MPT64 protein at various concentrations. A 1:10 dilution of 1mg/mL MPT64 was followed by serial (1:3) dilutions. MPT 64 dilutions were then analyzed using two concentrations of the purified MPT64 antibody (total IgY) and purified control IgY antibodies. This analysis showed the MPT64 antibody to be effective in detecting the purified MPT64 protein at concentrations as low as 0.6 ng/ml (Fig. 5, right). The two antibodies raised against the MTP64 protein (7759 and

7760) had similar responses to the antigen. A 1:100 dilution of the antibody was more effective in detection the lower levels of MPT64 than at 1:1000.

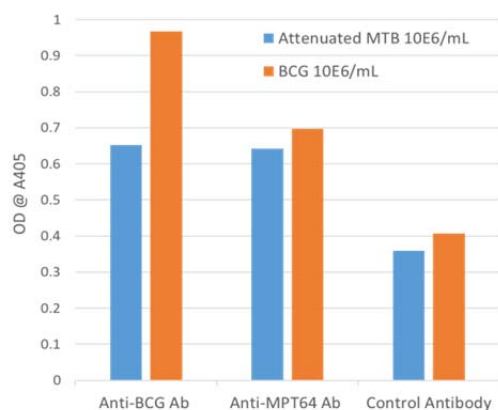


Figure 6. Binding of MPT64 antibody and BCG antibody at 28µg/mL to whole *Mycobacterium* cells.

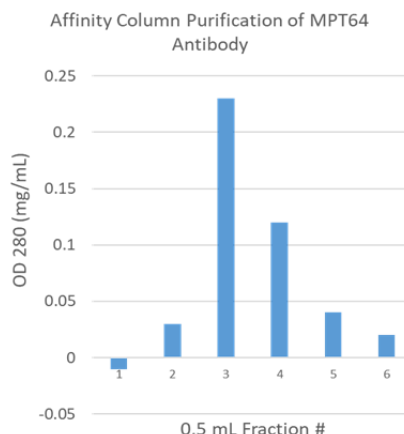


Figure 7. Affinity purification of MPT64 antibody using a column of immobilized MPT64.

IgY antibodies raised to BCG (vaccine strain of *M.bovis*) were also tested in comparison to the MPT64 antibodies. BCG was harvested from a 5-week growth at 37°C in 7H9 media, washed and resuspended in PBS and stored frozen at -80C. A 96-well filter bottom plate was used to test antibodies against whole cells, both BCG and the highly attenuated H37Ra strain of *M.tuberculosis*. Because MPT64 is secreted into the growth media, the filter assay should trap the cells but remove the free protein from this assay.

Fig. 6 shows that the MPT64 antibody binds to both the MTB and BCG whole cells, while the background (pre-immune IgY from the same hen) shows a much lower response. This response of MPT64 antibody to whole *Mycobacterium* cells may be due to the Complete Freund's Adjuvant used in raising the antibodies.

To reduce background binding, the MPT64 antibody was affinity purified using an affinity column of immobilized MPT64 protein. Fig. 7 shows the fractions of specific anti-MPT-64 IgY as they are eluted from the affinity column. Affinity purified antibody was collected in 0.5mL fractions and then concentrated and exchanged in PBS at a final concentration of 2mg/ml. Affinity purified antibodies will also create more concentrated specific antibody on the sensor surface.

The affinity purified antibody was again tested against BCG to determine the extent of background binding. To achieve this, an ELISA to MPT-64 as well as a filter EIA to BCG cells was performed. Fig. 8 shows that the affinity purified anti-MPT64 binds to antigen and gives a similar signal as the unpurified antibody.

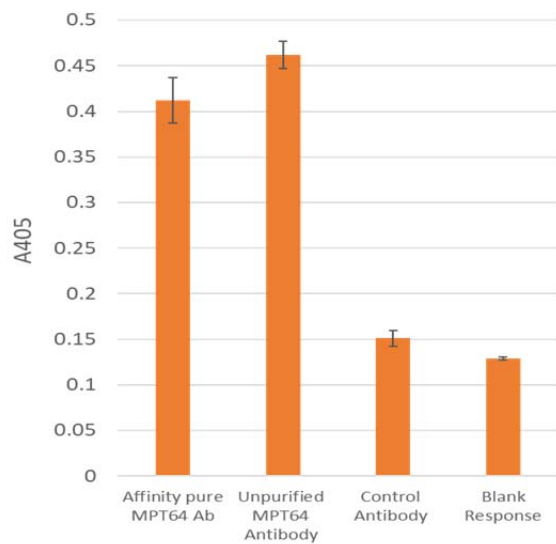


Figure 8. ELISA to MPT64

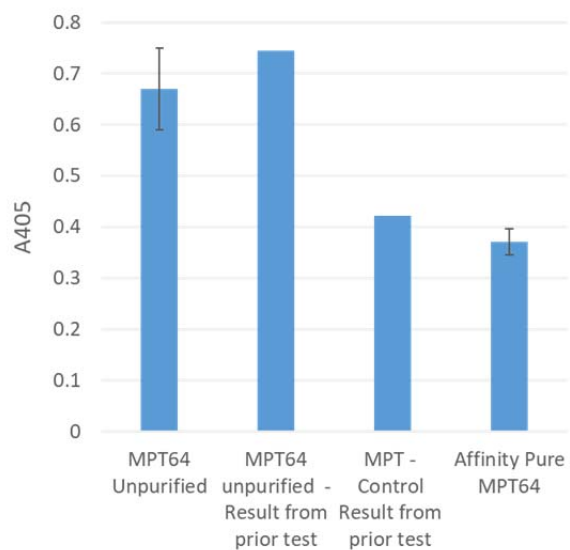


Figure 9. Filter EIA to BCG cells

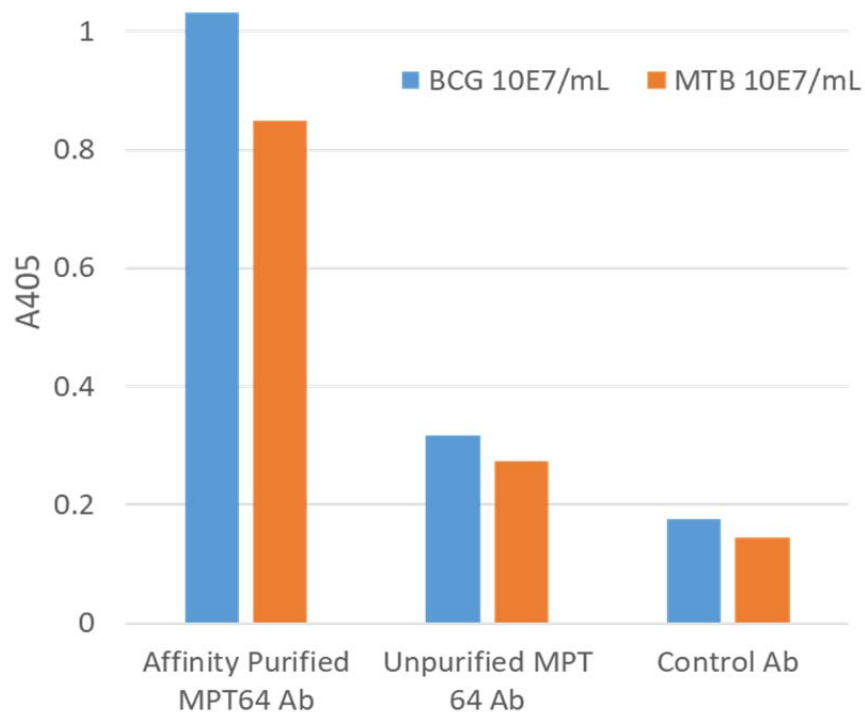


Figure 10. ELISA to incubated BCG and MTB H37Ra

Fig. 9 shows that the affinity pure MPT-64 antibody now has a lower response, comparable to control IgY (background) for BCG binding. Affinity purification appears to reduce or eliminate the binding of the MPT64 antibodies to BCG cells.

Testing the response to MPT64 from a filtrate of growing MTB cells would be ideal. However, frozen aliquots of BCG and MTB-H37Ra were used instead to test for extracellular MPT64. To test this, an aliquot of BCG and H37Ra cells was diluted to 1×10^7 cells/mL in PBS and incubated overnight at 37°C in an ELISA (protein binding) 96-well plate. Cells were then removed and the protein bound to the plate walls was assayed using both purified and unpurified MPT64 antibody. Fig. 10 shows these results, with the affinity purified antibody demonstrating a much higher response than with the unpurified antibody. The antibody was diluted 1:100 from a 2mg/ml purified or 20 mg/ml unpurified or control stock solutions. The results show a positive signal from the purified antibody for both BCG and H37Ra. Not all BCG strains produce MPT-64 (Byeon *et.al. Journal of Veterinary Science*, 16(1), 31, 2015), so it is difficult to assess whether this is a true MPT64 signal, but the increased response from the purified antibody over unpurified and control (pre-injection) antibodies is promising.

Task 2B: Fabrication of antibody immobilized sensors

The final fabrication protocols were:

The sensors were fabricated on polyethylene terephthalate (PET) films (Fig. 11a). Target cells and antigen were detected using a SWCNT sensor functionalized with polyethyleneimine (PEI) and antibodies (Fig. 11b and 11c). Interdigitated silver electrodes were stamped for resistive detection. When targets were bound on the sensor surface, the resistance decreased due to the electrostatic interaction.

For fabrication (Fig. 12a~21d), SWCNTs were dispersed in 1% SDS at a concentration of 5 mg/mL using an ultrasonic bath at room temperature for 3 hours. The SWCNTs were spin-coated onto a PET film at 6,000 rpm for 20 seconds. The SWCNT film was cured at 100°C on a hot plate for 10 minutes. PEI (0.1% in DI water) was coated on the SWCNT surface. Subsequently, the PEI-coated SWCNT film was cured at 100°C on a hot plate for 10 minutes. For silver electrode patterning, a Delrin[®] mold was machined by using an end mill. The stamp was made of polydimethylsiloxane (PDMS) cured in a mold at room temperature for 3 days. The PDMS stamp coated with silver ink (EMS CI-1001) was used to print silver electrodes on the PEI-coated SWCNT sensors. The sensors were cured on a hot plate for 1 hour at 80°C. A polyclonal IgY antibody (1.8 mg/mL) raised against MPT64 protein was physisorbed on the SWCNT surface in PBS for 24 hours in a refrigerator (4 °C). Subsequently, each sensor was cut with scissors by half to generate 2 sensors (Fig. 12e). A total of 24 sensors were fabricated on each 40×40 mm² PET film. Fig. 12e shows the images of a sensor composed of one pair of interdigitated electrodes. The silver electrodes having the gap size of 200~300 μm are connected with functionalized SWCNTs (Fig. 12f and 12g).

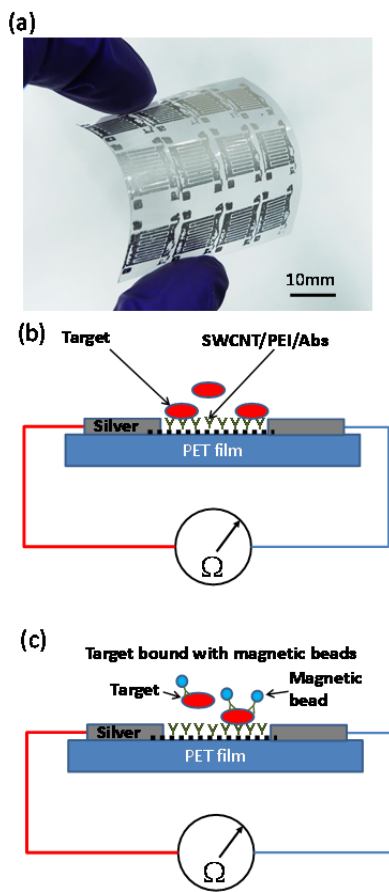


Figure 11 (a) SWCNT-based sensor on a flexible PET film. (b) Cross section of a resistive SWCNT immunosensor for detection from tongue swab samples (c) Test using magnetic beads for sputum samples.

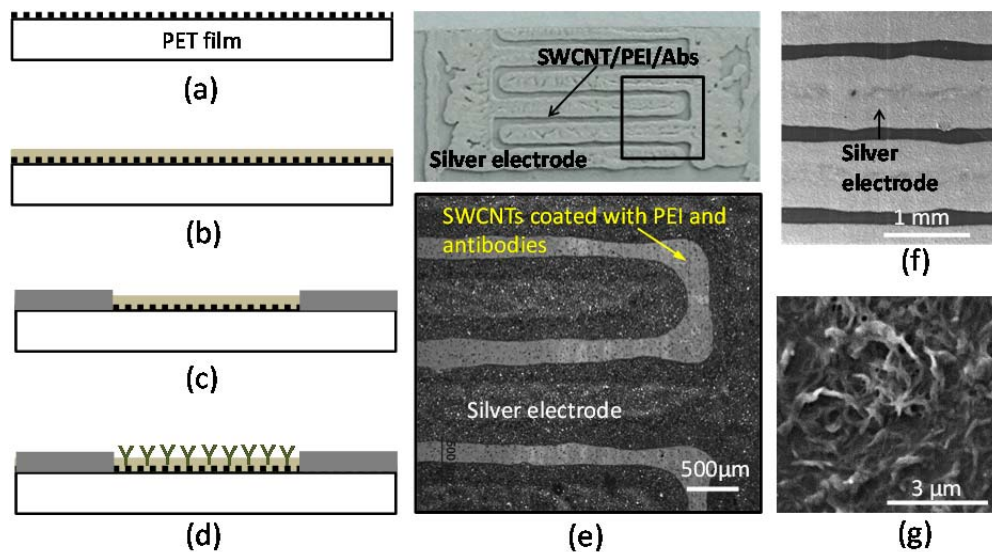


Figure 12 Fabrication process of a SWCNT-based immunosensor (a) Spin coating of SWCNTs on a PET film (b) Spin coating of PEI (c) Stamping of silver electrodes. (d) Antibody immobilization on the SWCNT/PEI film (e) Photo and optical microscope images of a SWCNT immunosensor (f) SEM image of SWCNTs between silver electrodes. (g) SEM image of SWCNTs.

In this sensor configuration, silver electrodes were stamped on SWCNTs in order to minimize the exposure of the interfacial area between SWCNTs and silver electrodes. Using this configuration, the oxidation of the silver electrode surface should not affect the resistive change for target detection, offering a uniform contact resistance and isolated the Schottky effect in the sensing mechanism. The electrostatic gating effect was the only mechanism that detected the target analytes.

Task 2C. Evaluation of sensor to sensor variation

To evaluate sensor-to-sensor variations for fabricated sensor, the detection of the target antigen was performed by I-V measurement (Fig. 13). The first step was to measure the baseline signal. One μl of antibody was then applied to the device and another measurement was collected. After the rinsing with DI-water for 10 seconds to eliminate excess antibody molecules that did not properly bind to the SWCNTs, I-V measurement was conducted again. For negative control, 10 μl of phosphate buffered saline (1x PBS) was incubated for 10 min and gently removed with a nitrogen gun for 1 min. The results showed that the current change (resistance change) was repeatable within the error range of 10%.

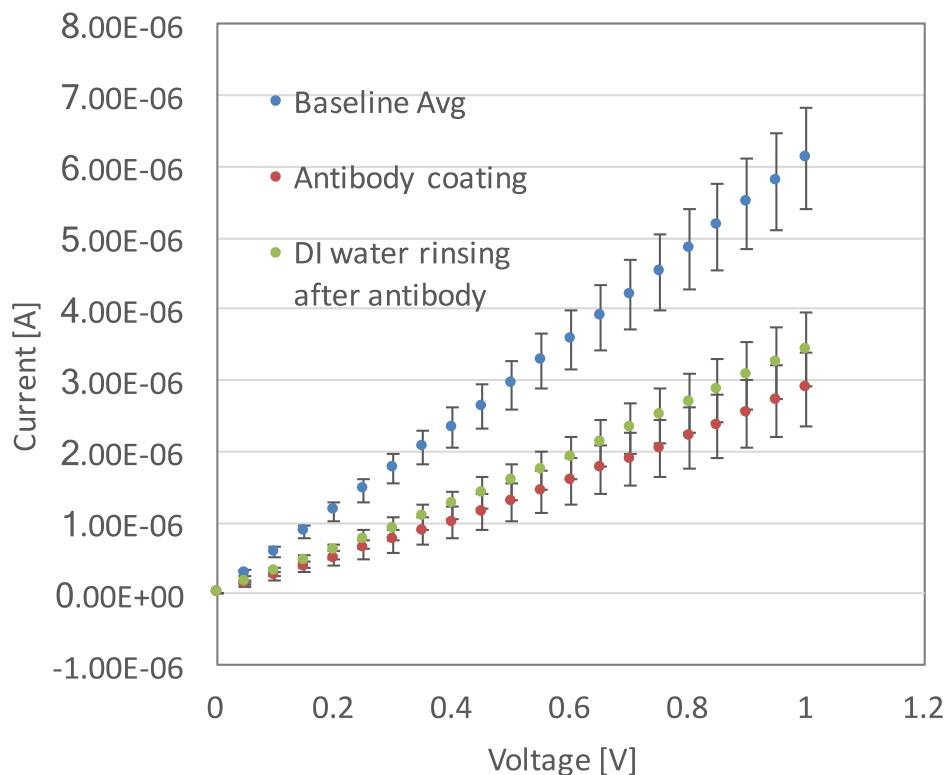


Figure 13. Sensor-to-sensor variations for fabricated device (n=8).

In the immobilization step, the binding between antibodies and PEI is ionic binding between negatively charged antibodies and positively charged amine groups. To confirm the function of antibodies, we used fluorescence-labelled BCG cells (10^7CFU/mL) to determine if the immobilized antibodies react with BCG cells. Fig. 14 shows

that immobilization of antibodies on PEI-coated SWCNTs captures BCG cells. White dots are the colonies/clumps of stained BCG cells. The binding was strong enough to withstand the rigorous washing step using PBS and deionized water. In summary, a very simple immobilization step of antibodies on sensor surface was achieved by using PEI-coated SWCNTs.

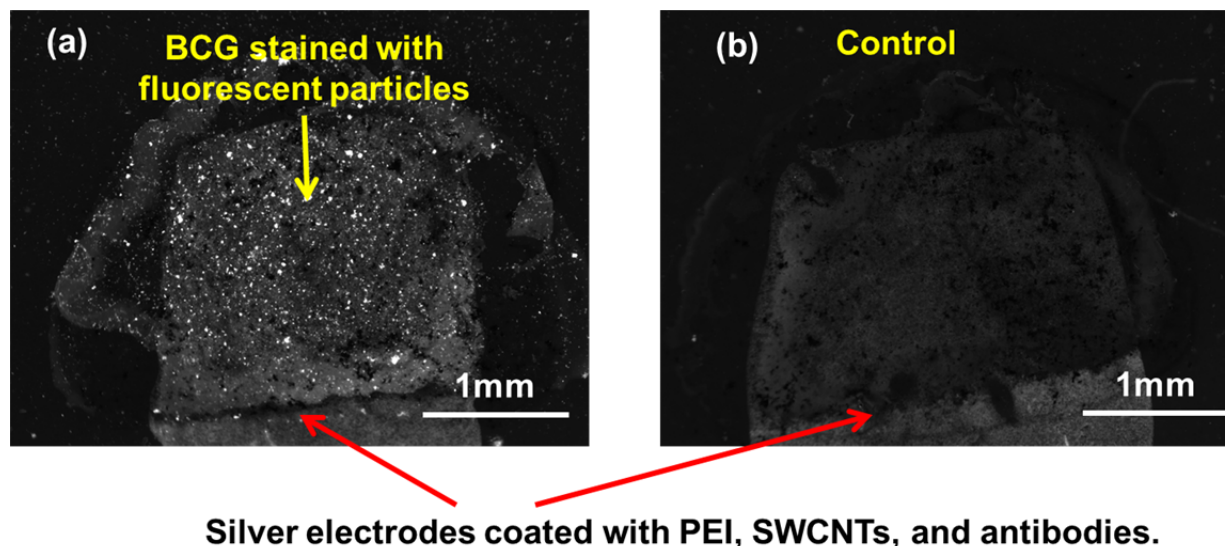


Figure 14. Fluorescence images showing the binding between BCG cells and MPT 64 antibodies. (a) Fluorescently stained BCG cells bound on to electrode surface. Electrode consists of silver/PEI/SWCNTs/antibodies. (b) Control electrode without stained cells.

Sensor characterization

After antibody immobilization, the resistance of SWCNT sensors increased upon binding of antibodies, ions, and hydrogen on SWCNTs. To study the contribution of resistance increase by the antibody immobilization step, the antibody concentrations varied from 0, 0.9, 1.8, and 4.5 mg/mL in PBS buffer. After the 24 hour incubation of the SWCNT sensors in each solution, the normalized resistance change was measured before and after antibody immobilization.

When the sensor was exposed to air out of the antibody solution, the sensor resistance started to decrease due to the environmental change and hydrogen desorption. Of the attempts to increase the signal to noise ratio, the incubation at 35 °C after antibody immobilization was shown to produce the best result. The sensor response for the target samples (*MTB* at 10^6 CFU/mL in PBS) compared to control (no *MTB* in sample solution) was tested after 5, 20, 40 and 120 minutes of incubation at 35 °C. The curing temperature was not increased over 35°C to avoid degrading the antibodies. In the curing step, the humidity ranged between 25 and 30%.

In the 24 hour antibody immobilization step, the sensor resistance was changed by the binding of hydrogen, ions, and antibodies. Fig. 15a shows the normalized resistance change of SWCNTs before and after antibody immobilization for antibody concentrations of 1.4, 2.8, and 7.0 $\mu\text{g/mL}$. The normalized resistance change of SWCNTs in PBS was 1.78 while that in antibody solutions varied from 2.04 to 2.12 on average. On average, the 70 % of the resistance change was attributed to hydrogen and ion bonding, and 30 % was from antibody binding.

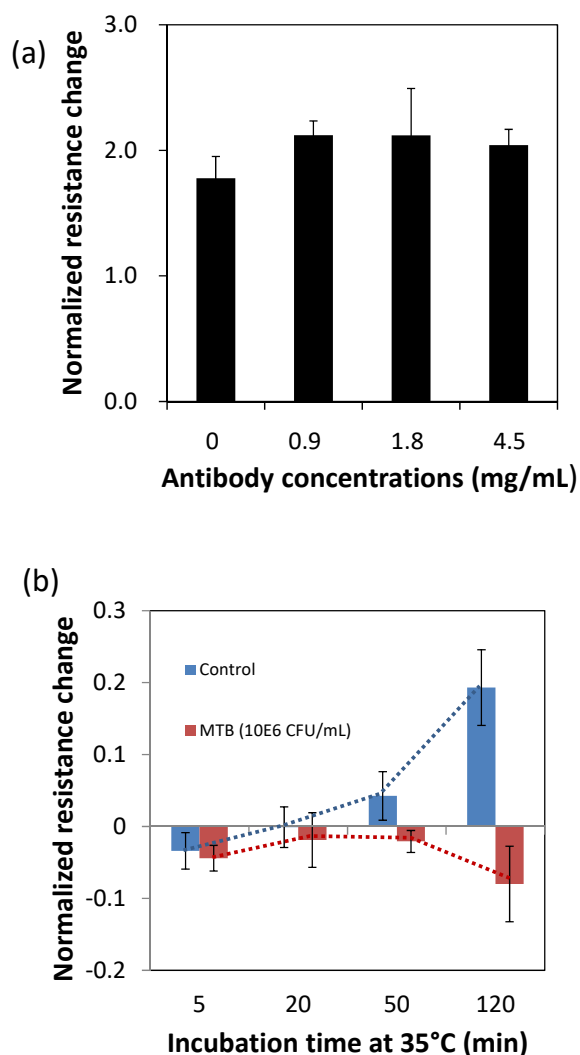


Figure 15 (a) Normalized resistance change before and after antibody immobilization on SWCNT sensors (N=4) (b) Normalized resistance change of a SWCNT sensor for control and *MTB* (10⁶ CFU/mL) in PBS (N=4). The sensors are tested after 5, 20, 40, and 120 min incubation at 35 °C.

To study the curing effect of a 35 °C step following antibody immobilization, the immunoassay using SWCNT sensors was tested after 5, 20, 40, and 120 minutes of incubation at 35 °C. Fig. 15b shows the normalized resistance change for *MTB* (10⁶ CFU/mL) at 35 °C. The control was negative at 5 min and gradually increased to the positive value. The normalized resistance of the positive *MTB* samples maintained slightly negative values and dropped to -0.08. When the control was compared with the positive cases, a signal could be differentiated for the samples of 40 and 120 min incubation at 35 °C. In the sensitivity, specificity and LLD experiments, the incubation time and temperature were maintained as 120 min and 35 °C.

Aims 2a (Task 3) Demonstrate the prototype device with pure samples

Task 3A: Preparation of cells and MPT 64

For the preparation of bacteria cells used to spike samples, a stock solution of cell suspension was added into Difco Middlebrook 7H9 Broth (BD Diagnostics, Sparks, MD) supplemented with 10% (v/v) ADC enrichment and

0.05% Tween 20. They were cultivated in a shaker incubator at 37°C until cultures were saturated. The cell growth was monitored by a UV photometer measuring optical density (A600). Both *MTB* and MPT64 were suspended in 1x PBS buffer. For *MTB*, various concentrations of *MTB* cells were suspended in PBS from 10^1 ~ 10^5 CFU/mL. MPT64 was also suspended in PBS from 0.1 ng/mL to 1µg/mL with 10 fold dilutions.

Task 3B: Demonstration of sensor performance for MTB (BCG) in PBS buffer

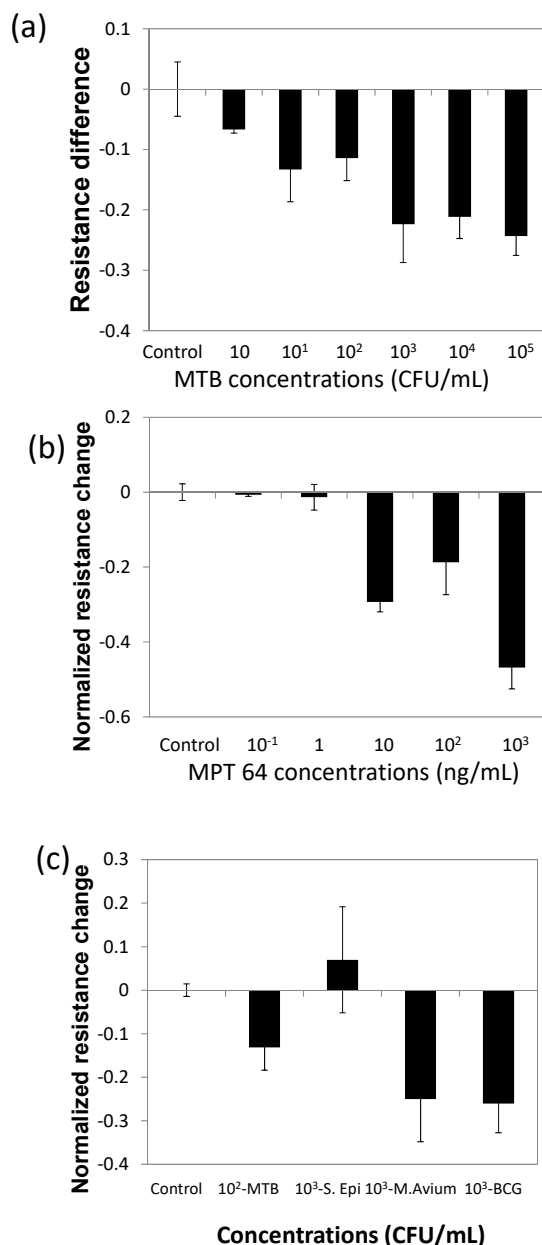


Figure 16 (a) Sensitivity test for *MTB* in PBS (N=4). (b) Sensitivity test for MPT64 in PBS (N=4). (c) Specificity test results for *MTB* (10^2 CFU/mL), *S. Epi* (10^3 CFU/mL), *M. Avium* (10^3 CFU/mL), and *BCG* (10^3 CFU/mL) (N=4).

Sensitivity and specificity tests

For sensitivity and specificity tests, both *MTB* and MPT64 were suspended in 1x PBS buffer. For *MTB*, various concentrations of *MTB* cells were suspended in PBS from 10^1 ~ 10^5 CFU/mL. MPT64 was also suspended in PBS from 0.1 ng/mL to 1µg/mL with 10 fold dilutions. 1 mL of each solution was supplied in each plastic cup where a

sensor was immersed for immunocomplex formation. After 10 min of the incubation, the sensor was rinsed with DI water. After the gentle blow dry with nitrogen, the resistance was measured. The resistance values before and after immunocomplex formation were R_0 and R_f , respectively. The normalized resistance change $[(R_f - R_0)/R_0]$ was computed to compare the signal from the control.

For specificity tests, the response for *MTB* (10^2 CFU/mL) was compared with *Staphylococcus epidermidis* (*S. Epi* at 10^3 CFU/mL), *Mycobacterium Avium* (*M. Avium* at 10^3 CFU/mL), and *BCG* at 10^3 CFU/mL. The bacterial samples were suspended in 1 mL PBS.

For sensitivity tests, various concentrations of *MTB* cells in PBS buffer were tested, as shown in Fig. 16a. The signal from 10 to 10^5 CFU/mL was compared to the control. In these tests, the normalized resistance change for the control was measured between 0.15 and 0.25. The average value of the normalized resistance for the control was shifted to 0 for convenience of reporting. The control signal was shifted down, while the detection signal was even further decreased. Despite the high sensitivity, the resistance change was not quantitative with respect to *MTB* concentration.

Task 3C: Demonstration of sensor performance for MPT64 in PBS buffer

When the dose-response test was conducted for antigen MPT64, the signal was detectable starting at 10 ng/mL (Fig. 16b). For the specificity test, the signal of *MTB* at 100 CFU/mL was clearly differentiated from the control and *S. epi* at 10^3 CFU/mL (Fig. 16c). However, *M. Avium* (10^3 CFU/mL) and *BCG* (10^3 CFU/mL) showed positive response due to the cross-reactivity to *Mycobacterium* strains, including NTM.

Aims 2b (Task 4). Demonstrate the prototype device with simulated sputum sample

Sputum processing: In this task, we aim to liquefy and decontaminate sputum samples for detection of target analytes. We tested two protocols for sputum liquefaction. The main purpose was to dilute sputum samples and to achieve homogeneous medium physically. The uniform ion concentration using the high ionic reagents will result in stable control signal of the sensors because electrostatic interaction is a key for the sensing mechanism. The total volume after adding reagents became 4 times the original sputum volume. The addition of reagents will offer a relatively uniform electrical resistivity to obtain more consistent measurement of resistance.

The two kinds of sputum samples tested were human sputum samples and synthetic sputum samples. Sputum samples were obtained from BioReclamation, Inc. The samples were deidentified from the company. Synthetic sputum was prepared according to Sanders *et. al.* (Sanders, N. N., Van Rompaey, E., De Smedt, S. C. & Demeester, J. Structural alterations of gene complexes by cystic fibrosis sputum. *American Journal of Respiratory and Critical Care Medicine* **164**, 486-493, doi:10.1164/ajrccm.164.3.2011041, 2001). Briefly, to a 21.74 ml buffer solution (85mM NaCl, 3 mM CaCl₂, 20 mM HEPES, pH7.4) was added 500 mg mucin (sigma #M3895), 91.35mg DNA (sigma #D1626), BSA 543.7 5 mg (sigma #A7030), 57.55 mg DPPC (sigma #P0763) and 8.1 mg DPPG (sigma #42627) and vortexed, creating a very viscous mucus like consistency.

To reduce viscosity and clarify the sputum, the following protocols were tested. 100 μL sputum was first mixed with 100 μL PBS followed by either 100 μL NALC (4 mg mL^{-1} N-acetyl-L-cysteine) or 100 μL 0.4M NaOH. 3mm glass beads were added to the tube and the solution was vortexed for 5 minutes. After vortexing a 4% SDS solution (sodium dodecyl sulfate, 100 μL) was added, followed by additional vortexing for 5 minutes for complete liquefaction. Liquefaction and clarity of sputum was tested by measuring OD at 600nm.

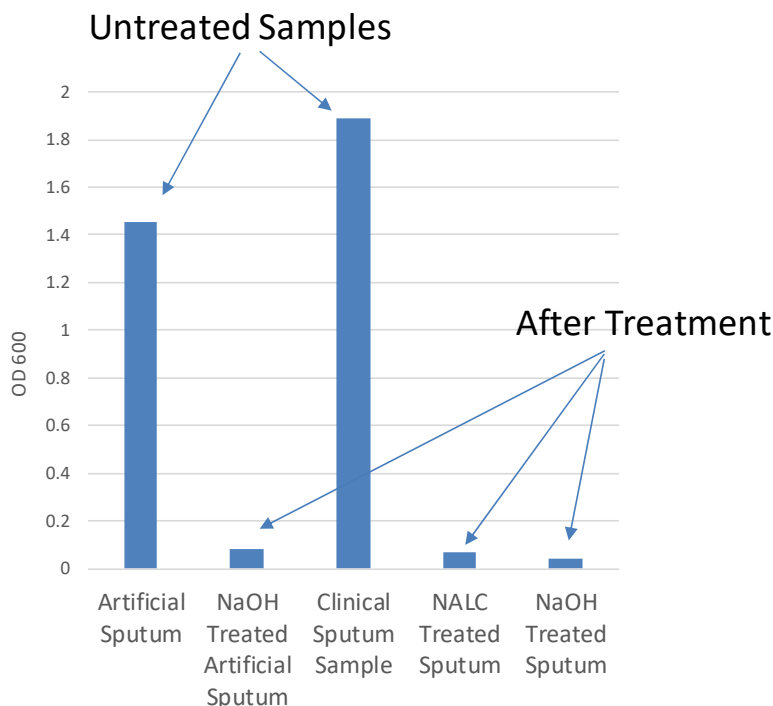


Fig. 17 shows the results of the OD measurement. The results show a significant reduction on OD600 after

Figure 17. OD 600nm Results of Sputum Processing. Both NALC +SDS and NaOH+SDS are effective in liquefying the sputum samples. Low OD measurement values show successful liquefaction of sputum samples.

treatment. The sputum samples were also significantly less viscous (a qualitative observation when pipetting the sample). Both the NaOH and NAC treatments worked to reduce the OD600, and the procedure worked on both the human samples as well as the artificial sputum samples. The artificial sputum samples were tested with NaOH only. Both sputum protocols will be used for detection of target analytes in sputum. In addition, a solution of Triton X-100 detergent was used in place of SDS and showed similar results to SDS containing samples, which may be better for antibody containing samples (preliminary data not shown).

The final detection protocols were:

Tongue swab sampling is a newer approach for obtaining *MTB* markers of infection. To evaluate LLD for *MTB* and MPT-64 in tongue swab samples, the swab samples were prepared by scraping tongue surface from deidentified volunteers (Fig. 18a). After the complete drying of swabs in air, the swab samples were immersed in 1 mL PBS for 20 minutes with gentle stirring. Subsequently, 500 μL of target analyte (*MTB* or MPT64) in PBS was mixed with 500 μL of the eluted swab solution. The 1 mL solution was used to test the LLD. The spiked concentrations of *MTB* ranged from 10 to 10^5 CFU/mL in steps of 10-fold dilutions. The concentrations of MPT64 ranged from 1 ng/mL to 10 $\mu\text{g/mL}$ with steps of 10-fold dilutions. For analysis, each sensor was incubated with a 1 mL sample solution for 10 minutes. Before and after target binding, the resistance was measured to compute a normalized resistance.

The test protocol for human sputum samples is described in Fig. 18b. Deidentified human sputum samples were obtained from BioReclamation, Inc. To reduce the viscosity and liquefy the sputum, 100 μL sputum was first mixed with 100 μL PBS followed by 100 μL -NaLc (4 mg mL^{-1} N-acetyl-L-cysteine). Also, 3 mm-glass beads and a 4% SDS solution (sodium dodecyl sulfate, 100 μL) were added to the mixture with addition of the targets. The mixture

was vortexed for 10 minutes with 60°C heating for complete liquefaction. This protocol was described previously (1).

For magnetic enrichment of *MTB*, 200 μ L of the 400 μ L-liquefied sputum samples were mixed with 10 μ L of magnetic beads suspended in 450 μ L PBS. After 20 minutes of gentle stirring and incubation, the magnetic beads were held with a magnet while the sample solution was gently aspirated. The magnetic beads were then washed with 1 mL PBS followed by magnetic separation. After rinsing, 500 μ L of PBS solution was used to suspend the magnetic beads bound to the target. Using this protocol, the LLD was evaluated for *MTB*.

To evaluate LLD for MPT64, the protocol was slightly modified. Sputum samples (100 μ L) were mixed with 100 μ L-NaLc and 100 μ L-4 %-SDS. MPT64 (100 μ L; 0.1~10⁴ ng/mL in 10-fold increments) of each concentration were spiked in the mixture. Without 60 °C heating to avoid protein damage, the dissipated sputum samples were mixed with the magnetic beads. The following procedure was the same as the *MTB*-sputum protocol.

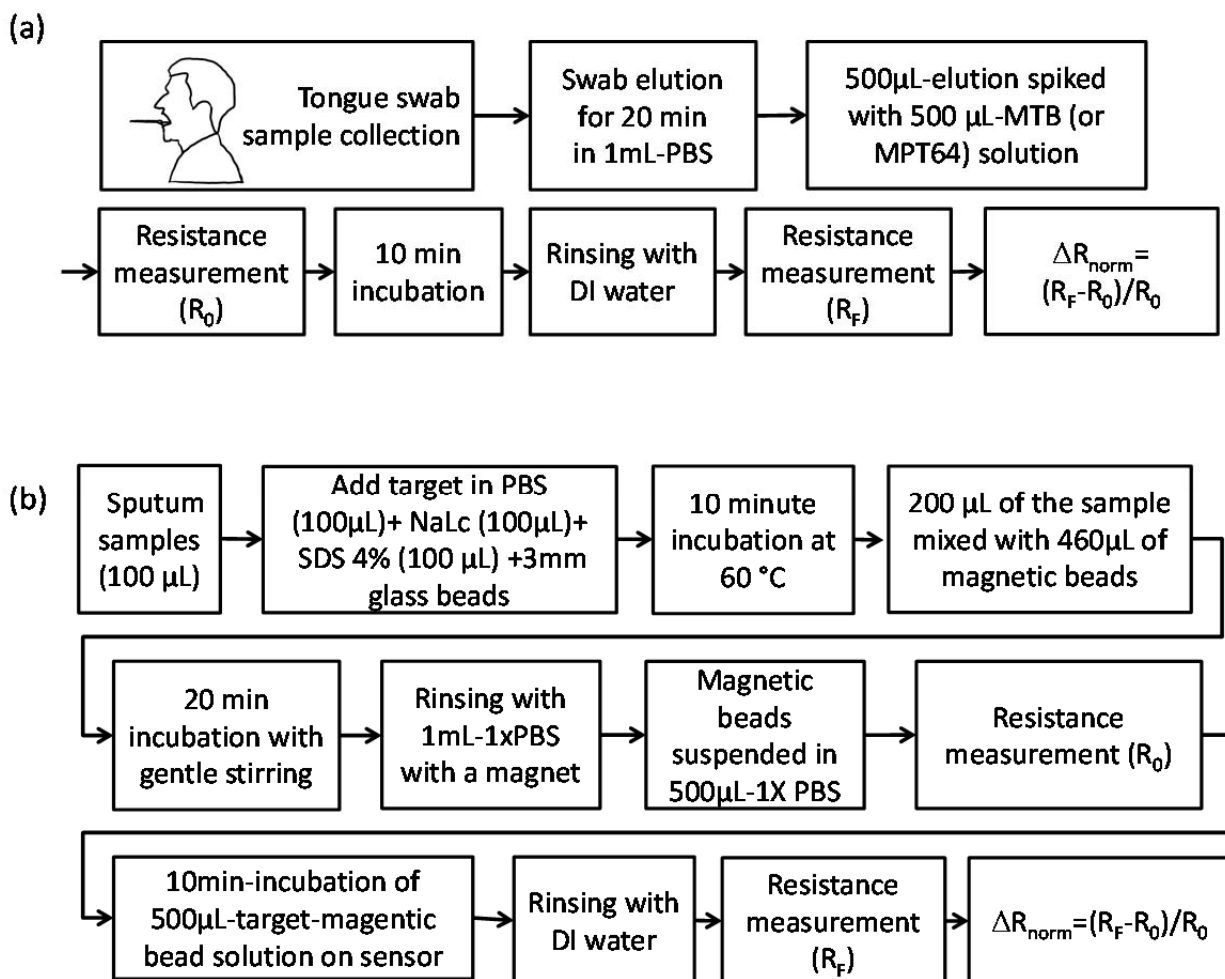


Figure 18 Sample preparation protocol and resistive detection procedure (a) Tongue swab samples (b) Sputum samples spiked with targets.

Preparation protocol for magnetic particles immobilized with antibodies: Carboxyl-functionalized superparamagnetic particles (Ocean Nanotech #MHP-100-01) were functionalized with anti-MPT64 antibody using a protocol modified from the bead manufacturer. Briefly, a 600 μ L aliquot of the 10mg/mL stock magnetic particles (MPs) was removed from the storage solution by applying a magnet for 5 minutes followed by careful removal of

the storage liquid with a pipette. The bead solution was then resuspended in a 0.5 mL solution of 0.4 M 1-ethyl-3-(3-dimethylaminepropyl) carbodiimide HCl (EDC) (Thermo Scientific # 22980) and 0.1 M N-hydroxysulfosuccinimide (NHS) (Thermo Scientific #24510) in double distilled (DDI) water and incubated for 15 minutes. The activated beads were then washed once by magnetic separation with 0.5 mL-DDI water (4°C), resuspended in 0.3 mL of the antibody solution (17 mg/mL antibody in DPBS), and reacted for 3 hours with mixing at room temperature. The bead-antibody solution was then washed three more times by magnetic separation in a storage buffer supplied by the manufacturer (10 mM PBS buffer with 0.02 % NaN₃, 0.01 % Tween 20, and 0.1 % BSA).

Test results using tongue swab samples and human sputum samples with magnetic beads

To evaluate the LLD for tongue swab samples, *MTB* at the concentrations ranging from 10 to 10⁵ CFU/mL were spiked into tongue swab samples. The detection limit was 10 CFU/mL (Fig. 19a). According to the dose-response test, the resistance change was not quantitative but qualitative. For the detection limit test using MTP-64 antigen, the LLD was 100 ng/mL, which was also qualitative (Fig. 19b). Given that tongue swab samples were replete with human cells, bacteria, and other microorganisms, these results also demonstrated the superior specificity of the SWCNT sensor.

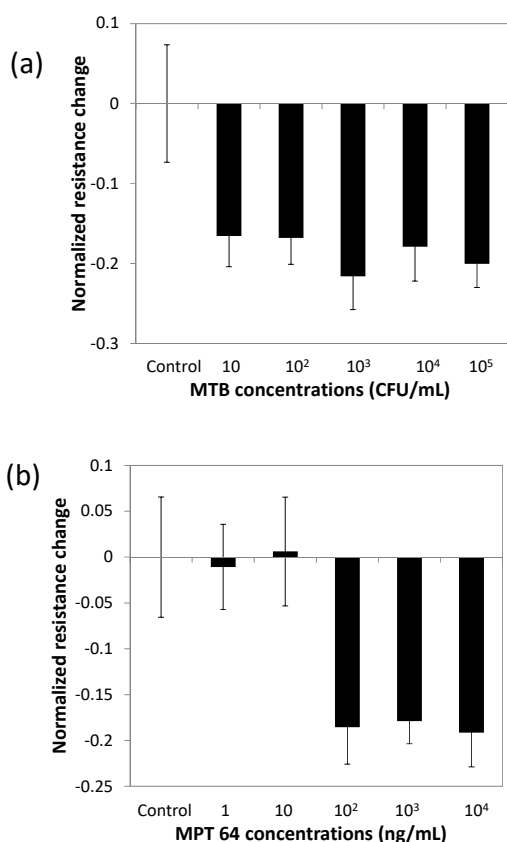


Figure 19 Lower limit of detection (LLD) tests for *MTB* and MPT64 (N=4) (a) *MTB* spiked in tongue swab samples (N=4) (b) MPT64 antigen spiked in tongue swab samples (N=4).

For the LLD for human sputum samples, *MTB* cells of 10 ~ 10⁴ CFU/mL were mixed with NaLc-treated sputum samples, and MPT64 from 0.1 ng/mL to 10⁴ ng/mL. The detection limit was 10² CFU/mL (Fig. 20a) for *MTB* and 100 ng/mL for the MPT64 antigen (Fig. 20b).

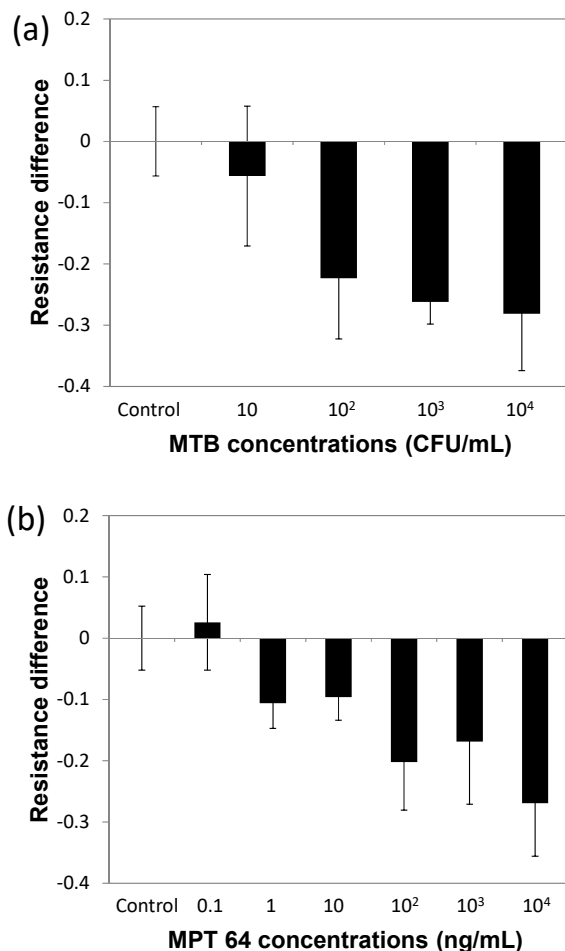


Figure 20 LLD tests for *MTB* and MPT64 spiked in human sputum samples. The targets are enriched with magnetic beads then detected with the sensors (a) *MTB* spiked in sputum samples (N=4) (b) MPT64 spiked in tongue swab samples (N=4).

To validate if the target cells were captured on a sensor surface, *MTB* cells (10⁶ CFU/mL) were observed on the SWCNT surface (Fig. 21a and 21b). Fig. 21c and 21d show the SEM images of *MTB* cells (10⁶ CFU/mL) bound with magnetic beads on the SWCNT surface. In the images, the white dots are crystallized ions from PBS. The qualitative not quantitative signal could be caused by the binding nature between bacterial cells and sensor surface. Considering the effective range of electrostatic detection as 10 nm, the nonuniform binding of target cells could result in a qualitative signal. The qualitative signal may also be related to the large gap size of 200 μm , explaining the saturation of the resistance change in the large gap size (2).

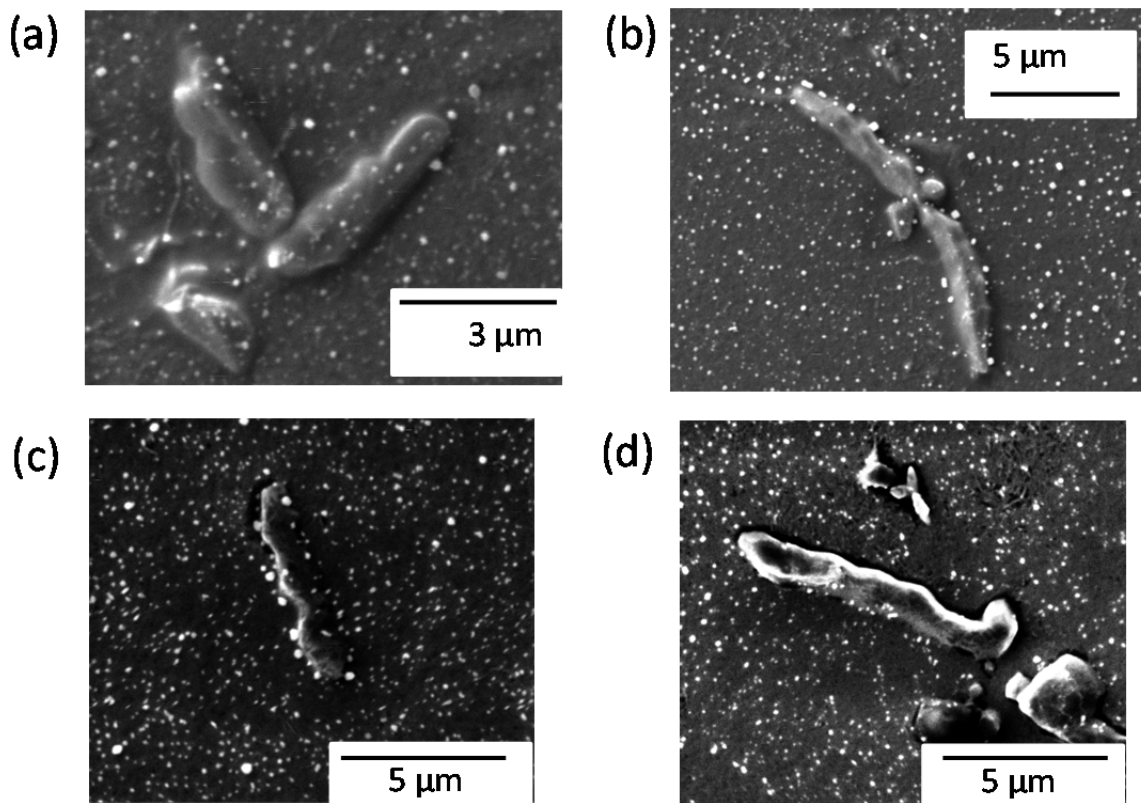


Figure 21 (a) and (b) SEM images of *MTB* cells (10^6 CFU/mL) in PBS. (c) and (d) SEM images of *MTB* cells (10^6 CFU/mL) captured with magnetic beads in PBS.

Use of PET films as sensor substrates can significantly reduce the material and manufacturing costs. Unlike the gold electrodes on silicon chips, the deposition of SWCNTs on silver electrodes resulted in unreliable contact resistance due to the oxidized silver layer. The rough surface of a PET film made the contact resistance higher (3). By stamping silver electrodes on a SWCNT film, a reliable resistance of a SWCNT sensor could be obtained. One of the major differences between silicon and PET substrates was the doping of SWCNTs on the PET film. While hydroxyl groups doped SWCNTs on oxidized silicon chips, carboxyl groups doped the SWCNTs on PET films. Although both substrates made SWCNTs p-type, the doping on a rough PET film could significantly change the SWCNT performance in combination with the PEI layer. For stable performance, the delicate control of the functionalization layers was critical. In the future, the addition of a control sensor next to a sensor will enhance the signal-to-noise ratio by compensating environmental factors including temperature.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?

The developed resistive immunosensor will have direct impact on the point of care (POC) diagnosis of tuberculosis (TB). The sensor detects MTB whole cells without cell culture and polymeric amplification. The simple but innovative method will require only low power allowing a portable sensing platform to be used for POC TB screening regardless of location and without a requirement for highly trained personnel. The developed immunosensor can replace insensitive and error-prone skin tests and reduce the screening time from 24 hours to 40 minutes for initial screening. Also, the typical target sample of sputum can be replaced with oral swab samples, which will result in a significant reduction of the initial screening time to just 30 minutes. In addition, the use of oral swab samples will make the developed resistive sensor more practical for field use due to its noninvasive and painless nature of sample collection. The easy sample collection protocol using tongue swab samples allows for screening of babies, children, seniors, or the injured. The further optimization and clinical validation will directly impact POC diagnosis for other infectious and cancerous diseases.

What was the impact on other disciplines?

The immuno-resistive sensor compatible with magnetic beads allows for accurate detection of other targets including nucleic acids, protein, viral particles, bacteria, and cells regardless of sample types and reagents. Considering the versatile performance, the developed assay will impact the screening and diagnosis of cancerous as well as other diseases.

What was the impact on technology transfer?

Based on the developed technology, a provisional patent was filed on May 28, 2019 by University of Washington. The title was "Flexible Resistive Single Walled Carbon Nanotube Sensor for Point of Care Screening of Diseases". The serial number was 62/853,492. The PI is searching for additional fund to test the immunoassay for a longer shelf life followed by clinical evaluation. The technology transfer office (CoMotion) at University of Washington investigates the background IPs and markets for the developed technology.

What was the impact on society beyond science and technology?

In this project, we have developed diagnostic assay that can be used at the point of care to rapidly and accurately diagnose TB. The developed sensor will be faster and at significantly lower cost than smear microscopy and PCR, and more accurate than skin tests. All this will help to provide patients with the right treatment without delay. In addition, we will be able to reduce the occurrence of false positives, which will spare the patient inconvenience, cost and potential toxicity from unnecessary drug treatments. Therefore, the immunosensor offers an affordable solution to address the current challenge of rapid, inexpensive and accurate TB diagnosis in low resource settings.

With respect to the technology, the major impact is on the resistive detection of target markers made of a thin plastic film. Although various biosensors made of single-walled carbon nanotubes (SWCNTs) have been developed, all the sensors have been fabricated using silicon chips. Few resistive SWCNT biosensors have been demonstrated on flexible plastic films. The thin plastic resistive sensor will significantly reduce the cost and screening procedure. The developed sensing technology will also impact the development of simple screening tools for cancer and infectious diseases.

5. CHANGES/PROBLEMS:

There was no significant change in the proposed plan. To successfully accomplish the proposed plan, we requested no cost extension of the project. The newly approved end date was October 30, 2019, which is one more year from the original termination date. The present annual report was described according to the changed plan.

▪ Changes in approach and reasons for change

In the original proposal, we proposed to test the sensing platform only for sputum samples. In addition to sputa, oral swab samples were tested due to their recent discovery as a convenient biosample source for TB diagnosis (4, 5).

Considering the convenience and easiness of the sample collection and detection steps, the TB screening using tongue swab samples is promising and pursued in our future effort.

▪ Actual or anticipated problems or delays and actions or plans to resolve them

The major challenges associated with the developed device were (1) to overcome the ion masking effect of SWCNT sensors and (2) to fabricate SWCNT sensors on a plastic film with silver ink electrodes for producing inexpensive film sensors.

(1) Ion masking effect of SWCNT immunosensors: For detection of targets, the change of electrical current and resistance was measured to identify the electronic modulation upon target binding. For SWCNT sensors, the electrical resistance could be changed at two locations; one was the interface between metal electrodes and SWCNTs (Schottky effect) and the other was the SWCNT surface (electrostatic effect). We tested more than 6 kinds of sensor designs, which failed due to the unpredictable change of resistance for both Schottky and electrostatic effects. SWCNT sensors were heavily doped with the high concentration of ions in PBS, which resulted in saturation of the doping level, and thus, insensitive SWCNTs. To overcome the ion masking effect, the sensor surface was rinsed with deionized (DI) water right after target binding. Interdigitated electrodes were fabricated to increase the surface area and to reduce the sensor resistance less than the buffer resistance. The unreliable Schottky effect was significantly reduced by stamping silver electrodes on a SWCNT surface. In addition, the SWCNT sensor exposed to water could be bound to protons, which made the resistance unpredictable. To avoid saturation of SWCNTs with protons, the fabricated sensor was incubated on a 40°C heater for 2 hours to release most protons bound on the SWCNT surface without degrading the IgY antibodies. All the efforts enabled the detection limit of 10 CFU/mL, which is one of the highest reported sensitivities among immunosensors.

(2) Fabrication of SWCNT sensors on a plastic film with silver ink electrodes: To fabricate resistive immunosensors on a PET film, metal electrodes were essential for the resistance measurement. The major problem with silver electrodes was the oxidative surface of silver materials, which made the contact resistance unpredictable. To address the challenge, interdigitated electrodes were stamped on SWCNT surfaces such that the interfacial region of silver electrodes was not exposed to air and directly contacted on the SWCNT sensor surfaces. In addition, the stamping step for silver electrodes was optimized to achieve uniform electrodes. In comparison to existing gold electrodes on silicon substrate, the fabrication method could reduce the sensor price by an order of magnitude with simpler manufacturing steps. All of our solutions will enable a very inexpensive but high performing sensing platform for POC diagnosis of TB.

- **Changes that had a significant impact on expenditures**

Nothing to report

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Not applicable.

- **Significant changes in use or care of human subjects**

Not applicable.

- **Significant changes in use or care of vertebrate animals.**

Not applicable.

- **Significant changes in use of biohazards and/or select agents**

Not applicable.

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

▪ **Publications, conference papers, and presentations**

1. S.-J. Kahng, C. Cerwyn, B. M. Dincau, J.-H. Kim, I. V. Novosselov, M. P. Anantram, and J.-H. Chung, Nanoink Bridge-induced Capillary Pen Printing for Chemical Sensors, *Nanotechnology*, 29, 335304, 2018.
2. Seong-Joong Kahng, "Wearable Carbon Nanotube Sensors: Fabrication and Applications for Bio/Chemical Sensors", PhD dissertation at University of Washington, June, 2019.
3. A provisional patent was filed by University of Washington: "Flexible Resistive Single Walled Carbon Nanotube Sensor for Point of Care Screening of Diseases" was filed on May 28, 2019, and has been assigned serial number 62/853,492.
4. Poster presentation: SJ Kahng, SD Soelberg, F Fondjo, JH Kim, CE Furlong, JH Chung, Carbon Nanotube-Based Thin-Film Resistive Sensor for Point-Of-Care Screening of Tuberculosis, Biomaterials Day by Society for Biomaterials, University of Washington, Seattle, WA, Dec 10, 2019.
5. Seong-Joong Kahng, Scott D. Soelberg, Fabrice Fondjo, Jong-Hoon Kim, Clement E. Furlong, and Jae-Hyun Chung, Carbon Nanotube-Based Thin-Film Resistive Sensor for Point-Of-Care Screening of Tuberculosis, in prepration for submission to a journal.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	<i>Jae-Hyun Chung</i>
Project Role:	<i>PI</i>
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0002-9861-8559
Nearest person month worked:	<i>1</i>
Contribution to Project:	<i>Chung has organized meetings among the project individuals, analyzed the data, and led the project.</i>
Funding Support:	
Name:	<i>SeongJoong Kahng</i>
Project Role:	<i>Research Assistance</i>
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	<i>12 months with 50% effort</i>
Contribution to Project:	<i>Mr. Kahng has fabricated and tested the sensors.</i>
Funding Support:	
Name:	Scott Soelberg
Project Role:	Research Scientist
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0001-8010-7753
Nearest person month worked:	2
Contribution to Project:	Mr. Soelberg has performed work in the area of antibody characterization and assay development
Funding Support:	
Name:	Clement Furlong
Project Role:	Co-PI
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0002-6489-7211
Nearest person month worked:	1
Contribution to Project:	Professor Furlong has served as PI for this subproject.
Funding Support:	

Name:	<i>Dr. Jong-Hoon Kim</i>
Project Role:	<i>PI</i>
Researcher Identifier (e.g. ORCID ID):	<i>0000-0001-6088-7676</i>

Nearest person month worked:	1
Contribution to Project:	<i>Dr. Kim has designed the experiments and managed the project activities and progress based on the planned timeline</i>
Funding Support:	
Name:	<i>Anwarul Karim</i>
Project Role:	<i>Graduate Student</i>
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	2
Contribution to Project:	<i>Mr. Karim has fabricated electrodes.</i>
Funding Support:	

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to report

- **What other organizations were involved as partners?**

Professor Gerard Cangelosi at the department of Environmental and Occupational Health Sciences advised to use oral swab samples to detect target analytes for tuberculosis diagnosis. Dr. Cangelosi discovered oral swab samples as the new sample source for TB diagnosis (4, 5).

8. SPECIAL REPORTING REQUIREMENTS

Nothing to report

References

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9. APPENDICES:

1. S.-J. Kahng, C. Cerwyn, B. M. Dincau, J.-H. Kim, I. V. Novosselov, M. P. Anantram, and J.-H. Chung, Nanoink Bridge-induced Capillary Pen Printing for Chemical Sensors, *Nanotechnology*, 29, 335304, 2018.
2. Seong-Joong Kahng, “Wearable Carbon Nanotube Sensors: Fabrication and Applications for Bio/Chemical Sensors”, PhD dissertation at University of Washington, June, 2019.
3. A provisional patent was filed by University of Washington: “Flexible Resistive Single Walled Carbon Nanotube Sensor for Point of Care Screening of Diseases” was filed on May 28, 2019, and has been assigned serial number 62/853,492.
4. Presentation in a conference: SJ Kahng, SD Soelberg, F Fondjo, JH Kim, CE Furlong, JH Chung, Carbon Nanotube-Based Thin-Film Resistive Sensor for Point-Of-Care Screening of Tuberculosis, Biomaterials Day by Society for Biomaterials, University of Washington, Seattle, WA, Dec 10, 2019.
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Nanoink bridge-induced capillary pen printing for chemical sensors

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Abstract

Single-walled carbon nanotubes (SWCNTs) are used as a key component for chemical sensors. For miniature scale design, a continuous printing method is preferred for electrical conductance without damaging the substrate. In this paper, a non-contact capillary pen printing method is presented by the formation of a nanoink bridge between the nib of a capillary pen and a polyethylene terephthalate film. A critical parameter for stable printing is the advancing contact angle at the bridge meniscus, which is a function of substrate temperature and printing speed. The printed pattern including dots, lines, and films of SWCNTs are characterized by morphology, optical transparency, and electrical properties. Gas and pH sensors fabricated using the non-contact printing method are demonstrated as applications.

Supplementary material for this article is available [online](#)

Keywords: single-walled carbon nanotubes, capillary pen, pH sensor, gas sensor, printing

(Some figures may appear in colour only in the online journal)

1. Introduction

Since the discovery of carbon nanotubes (CNTs), various patterning methods have been investigated to develop chemical and biological sensors. Early methods relied on direct growth on a substrate, assembly using an electric field, and self-assembly [1–3]. In the fabrication of wearable sensors, a non-contact printing is preferred to avoid potential damage to the substrate or existing layers. Thermal and piezoelectric inkjet printing methods [4–6] rely on thermal expansion or electromechanical vibration to eject droplets [5, 7]. Due to the droplet formation physics, inkjet printing is challenging for nanoink of low viscosity and high surface tension [8, 9] which often requires significant modifications of ink properties [10–12]. To achieve electrical conductance of the printed CNT patterns, the discrete nature of droplet deposition requires the ejector shift to connect the drops. In the printing

of biological materials, the fragile biomolecules may not be compatible with the high pressures, heat, and shear stresses associated with inkjet printing [9, 13, 14]. Electrohydrodynamic printing utilizes an electric field to control flow through a nozzle [15–17], but it is limited to a conductive and semi-conductive substrate. Stencil printing [18] deposits nanomaterial by spraying through a mask, which is limited to the designed masking pattern.

As an alternative, fountain pens [19–21], ball pens [22], and pencils [23, 24] have been demonstrated to draw nanomaterials. For fountain pens, the capillary force attracts ink into the tube and provides the pressure gradient to hold ink column inside. When the capillary tube is in contact with porous paper, the capillary pressure in a pen decreases due to the contact between the nib and the porous substrate, resulting in ink flow [25]. However, printing on an impermeable film is more challenging as the contact angle is much greater than 0°.

Moreover, any contact printing may damage the substrate [20] hindering multiple layer deposition required for complex sensor structures.

In this paper, a non-contact capillary pen printing method is presented. The technique is demonstrated by patterning single-walled carbon nanotubes (SWCNTs) via a nanoink liquid bridge. The non-contact printing method does not physically damage the substrate or previously deposited layers. A single pass printing is sufficient to obtain a measurable electrical resistance. The resistance and the optical properties are characterized for several print geometries: a dot, a line, and a film. The use of the printing method for fabrication of CNT based gas sensors and pH sensor is explored as a potential application.

2. Nanoink bridge-induced printing

The non-contact capillary method deposits nanoink through a liquid bridge forming between a capillary pen and substrate (figure 1(a)). A stylographic pen consists of a capillary nozzle and a rod-shaped ink stopper that assures nanoink seal when the pen is not used. During printing, two geometric parameters require control in order to maintain the capillary bridge integrity: the pen tip height (H) from the substrate and the advancing bridge contact angle ($\theta_{B,a}$) as illustrated in figure 1(a). When the liquid bridge is established, the ink flow rate depends on the pressure difference between the capillary pen reservoir and the substrate surface. In a static condition, $\theta_{B,a}$ is dependent on the substrate surface properties, its temperature, and ink properties. When the pen moves to the right, $\theta_{B,a}$ increases, and the recessing angle ($\theta_{B,r}$) decreases. As $\theta_{B,a}$ increases, the hydrostatic pressure on surface increases to reduce the ink flow. For low $\theta_{B,a}$, the pressure difference is maximized resulting in the high ink feed rate.

Figure 1(b) shows the printing setup consisting of an x - y - z plotter and control module. The printing direction is controlled by two step motors in x and y directions. Manual micropositioning stage sets the Z -coordinate (pen tip height; H) necessary to form a nanoink bridge. A camera with a microscopic objective lens monitors the condition of the liquid bridge for a feedback control. The substrate temperature is controlled via closed-loop by a thermocouple as a sensor for a heating stage.

Figure 1(c) illustrates the printing procedure. First, the nib is pressed in the axial direction, and nanoink is released due to capillary action. Second, upon the release of the nanoink, the pen is withdrawn from the substrate to $H = 100\ \mu\text{m}$; nanoink bridge forms between the pen tip and the substrate. When the pen moves to the right, $\theta_{B,a}$ increases and $\theta_{B,r}$ decreased. Finally, the pen is retracted to stop the print. Figure 1(d) shows a typical example of the printed pattern using the nanoink bridge printing on a polyethylene terephthalate (PET) film. A PET film is an appropriate substrate for chemical sensors because it is chemically resistant, transparent and mechanically robust.

3. Experimental methods

3.1. Characterization of printing method

To study the printing characteristics, a dot, a line and a film of SWCNTs were printed at various substrate temperatures and printing speeds. Temperature and printing speeds were controlled to obtain uniform line width and consistent electrical resistance because contact angle, viscosity and evaporation effect were critical factors to determine the printing quality. Nanoink was prepared by suspending SWCNTs ($5\ \text{mg ml}^{-1}$) in 1% sodium dodecyl sulfate (SDS) by sonication. In the suspension, the supernatant was used as nanoink. The advancing contact angle ($\theta_{B,a}$) on a PET film was measured for various substrate temperatures and printing speeds. The morphology of the printed patterns and the electrical properties were characterized. The nominal diameters of the capillary pens were 100, 300, and $700\ \mu\text{m}$. The outer diameters of the pen nib (D_o in figure 1(a)) were 225, 375, and $790\ \mu\text{m}$, respectively (supplementary information; figure S1 is available online at stacks.iop.org/NANO/29/335304/mmedia). The outer diameters determined the minimum line width in printing as the meniscus attached to the outer dimension of the nib. In the paper, the nominal diameters are used hereafter. The printer was located in a laminar flow hood. The temperature and the relative humidity in the chamber were $26 \pm 1.0\ ^\circ\text{C}$ and $35 \pm 2.0\%$, respectively.

3.1.1. Dot printing. A single dot was printed at various substrate temperatures $20\ ^\circ\text{C}$, $40\ ^\circ\text{C}$, $60\ ^\circ\text{C}$, $80\ ^\circ\text{C}$, and $100\ ^\circ\text{C}$ using a $300\ \mu\text{m}$ diameter pen. The pen reservoir was filled with SWCNT-ink and then installed on a printer. The pen was pressed and then withdrawn to $H = 100\ \mu\text{m}$ to form a nanoink bridge. To study the evaporation characteristics, the holding time was controlled at 1, 5, and 10 s at each temperature. The contact angles (θ_B) were measured for each case by using the camera images in the printer. The height profile of the dots was scanned by a profilometer (Alpha-step D-300 stylus profiler, KLA-Tencor Corporation).

3.1.2. Line printing. To print a line, numerical control (G-code) was used to control x and y directional step motors. A $100\ \mu\text{m}$ capillary pen was used. After the nanoink bridge formed at a $100\ \mu\text{m}$ gap, a straight line was deposited on a PET film. Printing speed was set in the range from 0.2 to $10\ \text{mm s}^{-1}$, and the temperature was controlled from $20\ ^\circ\text{C}$ to $100\ ^\circ\text{C}$. The line width and $\theta_{B,a}$ were measured for each speed and temperature. After printing, a silver paste was applied to both ends of the line to form electrodes. A picoammeter (6487 Picoammeter/Voltage Source, Keithley Instruments) was used to measure current-voltage (I - V) characteristics of the printed line. The printed line was imaged by an optical microscope (Olympus BX-41, Olympus, Gaithersburg, MD, USA) and scanning electron microscopy

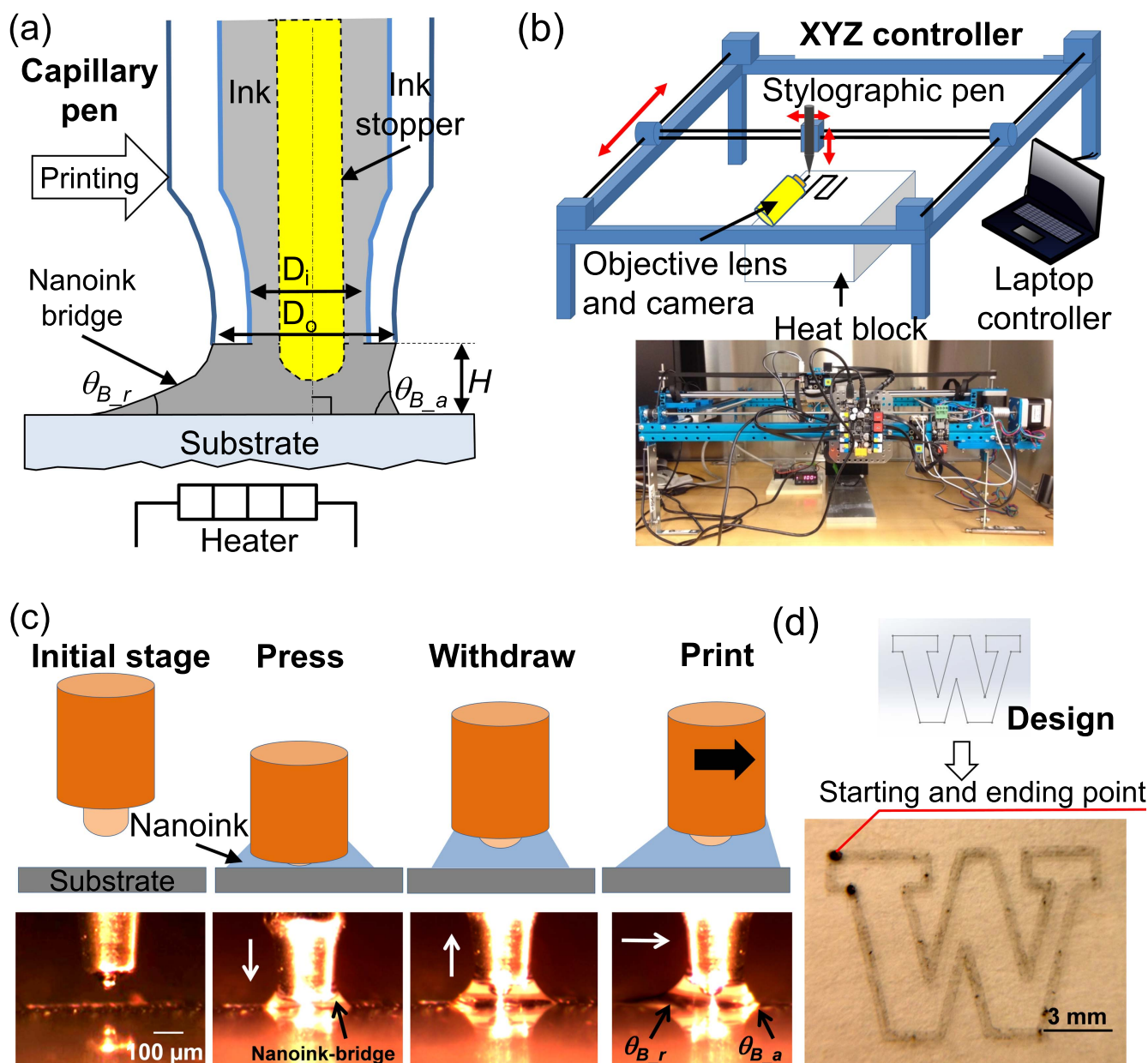


Figure 1. Nanoink bridge-induced capillary printing (a) printing concept (b) schematic of an XYZ plotter installed with a heating stage and a camera system. The top image shows a printing system, and the bottom is a photograph of the setup. (c) Nanoink bridge-induced printing using water ink on a PET film. The ink is released by pressing the stopper. Upon withdrawal by $100\ \mu\text{m}$, an ink bridge forms. The advancing contact angle increases as the pen moves from left to right. (d) W-pattern printed by SWCNT-ink at $80\ ^\circ\text{C}$ at $1.2\ \text{mm s}^{-1}$. The top image shows a design.

(SEM) in order to characterize the morphology of SWCNT lines.

3.1.3. Film printing. Film printing was achieved by drawing continuous lines in a linear hatch pattern covering the area of $15 \times 15\ \text{mm}^2$. The printing speed was set for $2.5\ \text{mm s}^{-1}$, and the substrate temperature was varied from $20\ ^\circ\text{C}$ to $100\ ^\circ\text{C}$. The nominal diameter of a capillary pen was $700\ \mu\text{m}$. To achieve complete coverage, the pen was shifted by $600\ \mu\text{m}$ for each pass (total of 25 parallel passes). Optical transparency measurements were performed by transmission optical microscopy (Olympus BX-41, Olympus, Gaithersburg, MD, USA). The transparency was computed as a ratio of the

transmitted white light intensity through the printed area over the non-printed area. The sheet resistance was measured using a custom 4-point probe measurement system (supplementary information; figure S2).

3.2. Doping effect

The electrical characteristics of the SWCNT lines were studied for doping polyethyleneimine (1% PEI, Fluka). The printing sequence was varied for PEI and SWCNTs. In one case, SWCNT lines were printed first, followed by PEI deposition (SWCNT/PEI). In the other case, the order was reversed. The SWCNT lines were printed on top of PEI (PEI/SWCNT).

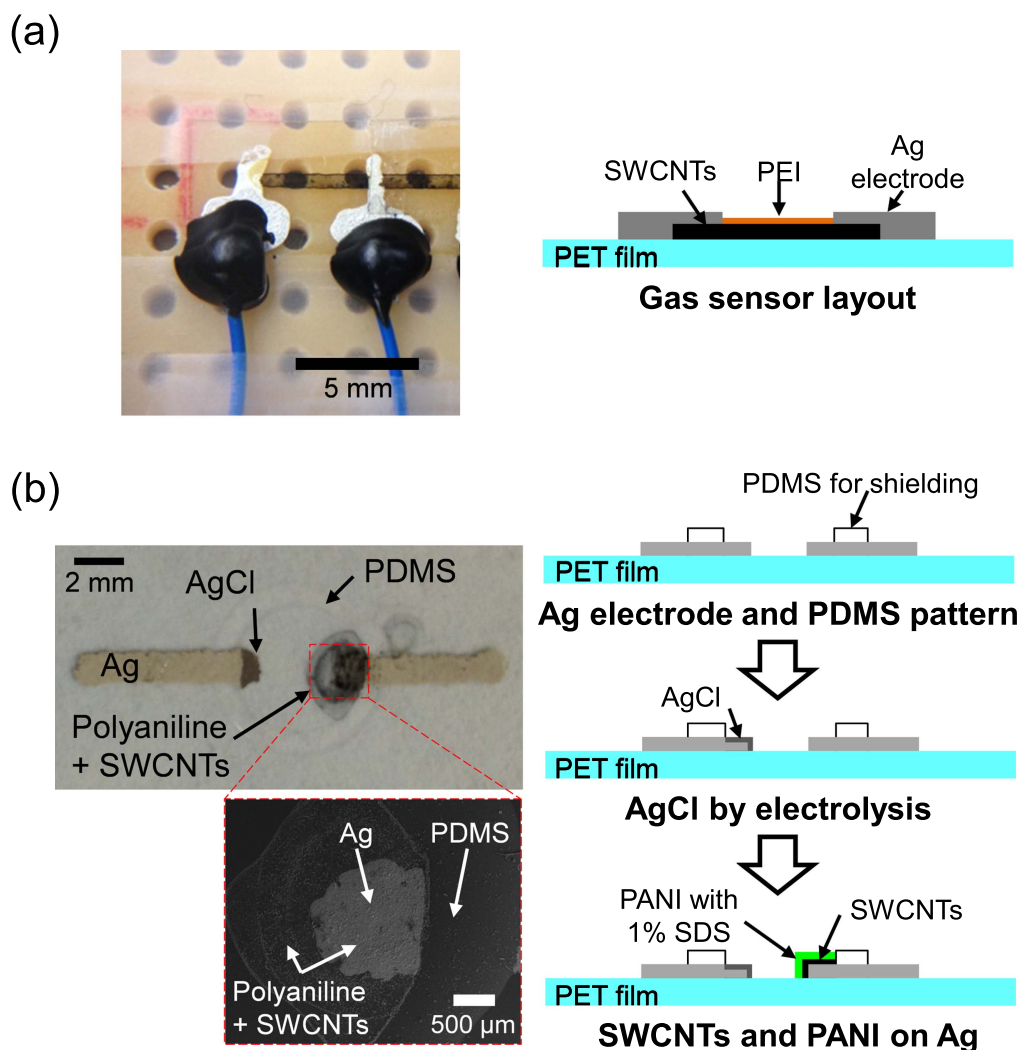


Figure 2. Fabrication steps (a) images and cross-section of a SWCNT-gas sensor (b) optical and SEM images and fabrication steps of a SWCNT-pH sensor.

The printing conditions were 80 °C with printing speed of 0.83 mm s⁻¹ where a stable line patterning could be obtained. Silver electrodes were patterned on the SWCNT lines. For both cases of SWCNT/PEI and PEI/SWCNT depositions, PEI solution (1 μl) was dispensed by 1, 2, and 3 times in order to analyze a doping effect and electrical stability. After each deposition, PEI was cured in a convection oven at 100 °C for 1 h. *I*-*V* characteristics were measured for all cases.

3.3. Sensor fabrication

3.3.1. Gas sensor. To fabricate a gas sensor, SWCNT electrode was printed as a line using a 300 μm diameter pen (figure 2(a)). The substrate temperature was held at 80 °C, and printing speed was 0.83 mm s⁻¹. Silver ink was deposited on both ends of the SWCNTs for electrical connection. The SWCNT electrode was functionalized by depositing 1 μl drop of 1% PEI, which was cured for 1 h at 100 °C in a convection oven. A second SWCNT electrode was coated with 1 μl drop of 1% Nafion (Nafion 117 solution, Sigma-Aldrich Co, LLC.) and cured for 1 h at 120 °C in a convection oven. Both

sensors were exposed to two different concentrations of NO₂ (8 and 22 ppm). The sensors' resistance was measured and recorded by a multimeter (287 True RMS Multimeter, Fluke Corporation).

3.3.2. pH sensor. Silver electrodes were screen-printed on PET film (figure 2(b)), which was cured at 100 °C for 10 min (supplementary information; figure S3). Polydimethylsiloxane (PDMS; Sylgard 184 silicone elastomer, Dow Corning Corporation) was stamped in a ring shape to hold analyte solution inside the ring. The PDMS was cured at 75 °C for 1 h in a convection oven. One of the silver electrodes (cathode) was modified to form an AgCl layer by electrolysis (1.5 Vdc for 1 min in 1 M HCl solution). For the other electrode (anode), SWCNT lines were printed on silver electrodes with speed of 0.83 mm s⁻¹ using a 300 μm diameter pen that covered the entire silver electrode surface. The printing temperature was 80 °C. The printing was performed like film printing to cover the square area of 1 mm². The printing conditions were 80 °C. The SWCNT printed electrode was cured for 10 min on a 100 °C hotplate. Polyaniline (5 mg ml⁻¹; PANI; emeraldine

salt, Sigma-Aldrich Co, LLC.) suspended in 1% SDS was deposited on top of the SWCNT electrode using two 1 μ l drops, followed by curing at 120 °C for 1 h. The voltage between the AgCl electrode and PANI electrode was measured using an Arduino circuit for standard pH solutions of pH 4, 7, and 10 (Omega Engineering, Inc.). Since the circuit measured the voltage difference ranging 0–3 V, a 1.61 V AA battery was serially connected to shift the voltage potential above 0 V in the control pH ranges (pH 7–10).

4. Results and discussion

4.1. Characterization of printing method

4.1.1. Dot printing. The section aims to characterize dot printed patterns at various temperatures and holding times. In the results, 60 °C showed the dot diameter to the nib diameter ratio of 1 with the uniform distribution of the SWCNTs due to a reduced coffee ring effect.

A circular dot printed pattern formed when a capillary pen was pressed and retracted to 100 μ m on a PET film for 1, 5, and 10 s (figure 3(a)). As the temperature increased, the ratio of the dot diameter to the outer nib diameter decreased and approached unity (figure 3(b)). The dot size reduction was attributed to the increase of the static contact angle (θ_{B-a}) and the pinning at the meniscus due to ink evaporation on the hot surface. As the substrate temperature increased, the surface tension and the viscosity decreased, however, the evaporation rate increased θ_{B-a} (figure 3(c)). As θ_{B-a} approached 90°, the meniscus edge was pinned yielding the ratio of unity. As the substrate temperature increased, the drop spreading on the substrate was reduced suggesting the effects of the increased evaporation rate at the drop edge. When the pen was withdrawn from the substrate, evaporation times decreased from 33 to 1 s as the temperature increased from 20 °C to 100 °C (supplementary information; figure S4).

Profilometer measurements of the dot depositions show that a coffee ring effect is present for a higher substrate temperature (figure 3(d)). The greatest deposition height occurred for prints with the surface temperature of 100 °C. During the deposition, SWCNTs were continuously delivered to the edge of the ink bridge where the liquid evaporation rate was the greatest, resulting in an increased local concentration of the SWCNTs in the solution, thus their thickest deposition at the dot edges. At room temperature, relatively uniform distribution of SWCNT-ink was observed as the ink flow rate, and its deposition rate was balanced by the evaporation rate.

4.1.2. Line printing. The section aims to characterize printed lines at various temperatures and printing speeds. In the results, 60 °C and 80 °C showed uniform line width under the printing speed of 2.5 mm s⁻¹ with a reduced coffee ring effect.

Microscopic observation of the printed line patterns shows that three distinct printing regions exist: (region 1) print line width decreases with the increase of print speed in

the low-temperature prints (20 °C and 40 °C); (region 2) line width is constant at medium temperature (60 °C and 80 °C); (region 3) line width increases with print speed—at high temperature (100 °C) and lower speeds. The trend is due to changing fluid properties and θ_{B-a} .

Region 1 was observed at the lower temperature conditions (20 °C and 40 °C) and medium temperature (60 °C) at higher print speeds; the line width reduced with the increase in print speed and the increase of the substrate temperature (figures 4(a) and (b)). At room temperature, the normalized line width, which is the ratio of the printed line width to the actual outside diameter of a Rotring pen, was reduced from 2.5 to 1 as the print speed increased from 0.2 to 2.5 mm s⁻¹. The nominal and actual diameters are given in the supplementary information (figure S1). At the low print speed, low contact angle and low shear stress, the line width was not uniform, indicating the unstable behavior of the capillary bridge. As the print speed increased both shear stress and contact angle, the bridge was stabilized, resulting in the stable printing conditions exhibiting constant line width and lower flow rate, which was also consistent with the increased sheet resistance. The reduction of the line width suggests that (i) the increased θ_{B-a} (figure 4(c)) causes the increase in the contact angle on the sides of the droplet parallel to the nib motion due to the surface tension, (ii) capillary bridge elongated along the printing direction with the shear stress. For the lower temperature condition, the prints failed at the speeds >2.5 mm s⁻¹, as θ_{B-a} approaches 90°.

Region 2 was characterized by relatively constant line width independent of the print speed at 60 °C and 80 °C. At the higher temperatures, the ink viscosity reduced resulting in the lower local shear stress allowing the ink to flow more uniformly at the larger contact angle. Experimentally the print speed increasing from 2.5 mm s⁻¹ to 10 mm s⁻¹ did not compromise the bridge stability though the advancing angle exceeded 90° for the faster prints. The high θ_{B-a} resulted in the high and stable contact angles on both sides of the bridge.

Region 3 was characterized by the increase in the line width as print speed increased at $T = 100$ °C and print speed below 2.5 mm s⁻¹. It is speculated that the effect was caused by the high rate of ink evaporation at the low print speeds as the liquid in the bridge approached its boiling point. As the speed increased, the time allowed for evaporation reduced. The ink was delivered to the substrate more effectively resulting in wider print line. At the higher speeds (>2.5 mm s⁻¹), the normalized line width reduced, which was consistent with the increase of θ_{B-a} as shown in figure 4(c).

Related to the optimization of the print conditions: at the temperatures greater than 60 °C, SWCNTs could be printed as $\theta_{B-a} > 90^\circ$. At the temperature of 80 °C and 100 °C, a stick-slip effect was observed at the advancing meniscus of a nanoink bridge. The beach mark pattern in figure 4(a) and an SEM study were consistent with the stick-slip effect (supplementary information; figure S5). Similar to the dot printing, the printed lines showed a coffee ring effect at the temperature >60 °C while a flat profile was observed at the temperature <60 °C. The thickness of the printed line was

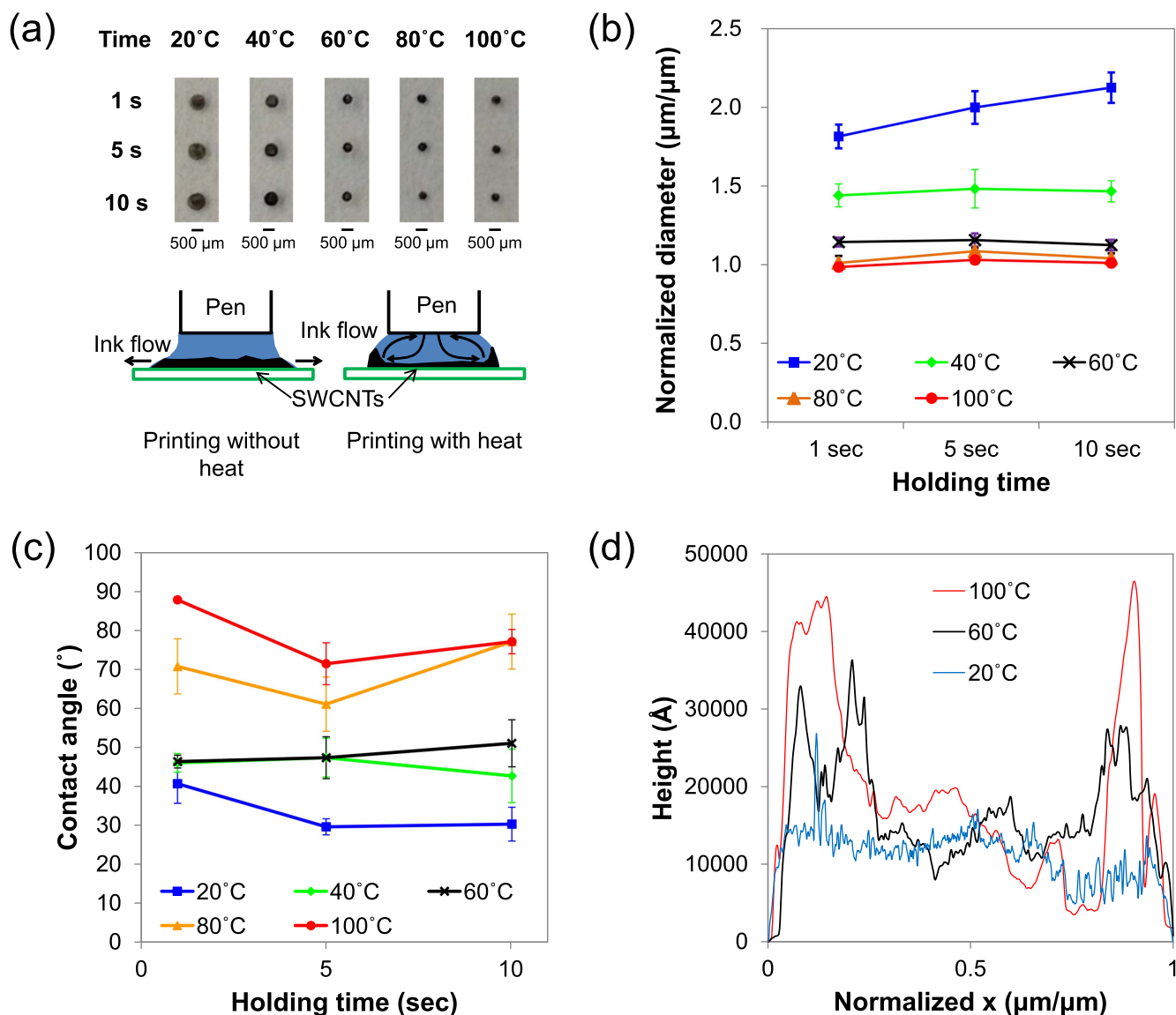


Figure 3. Dot printing (a) dots printed at surface temperature 20 °C–100 °C with holding time of 1, 5, and 10 s. Scale bar: 500 μm (b) dot diameters normalized by an outer nib diameter (c) contact angles according to holding time (d) dot profile at 20 °C, 60 °C, and 100 °C (1 s holding time).

well below 1 μm, which could not be measured by a profilometer or an atomic force microscope because of the relatively rough PET surface. The formation of the coffee ring could be observed by the higher contrast in an optical microscope and SEM (supplementary information; figure S4).

For electrical characterization of the prints, a silver electrode was patterned at the ends of a printed line. In comparison to 4-point probe measurement, the contact resistance using the 2-silver electrodes was only $9.6 \pm 0.6\%$, which was consistent throughout multiple measurements ($N = 6$). The I – V characteristics showed a linear trend due to the metallic SWCNTs in the printed lines. The sheet resistance of the lines became larger as both temperature and speed became higher (figure 4(d)). With the increase of θ_{B_a} , the smaller pressure difference reduced the flow rate of SWCNT-ink, which increased the sheet resistance.

Overall, the increase in the print speed yielded the greater sheet resistance as a result of higher contact angle and reduced flow rate. At the higher speed, the contact angle increased the pressure on the substrate, which resulted in the smaller nanoink flow rate thus the reduction of SWCNTs deposition and greater transparency of the print (supplementary information, figure S6). Unlike in the inkjet printing, both desired transparency and electrical resistance could be obtained with single print, which shows the advantage of the nanoink bridge-induced capillary printing.

4.1.3. Film printing. The section aims to characterize two-dimensional printing at various temperatures. In the results, 60 °C showed relatively high optical transmission and low sheet resistance.

For film printing, the area of $15 \times 15 \text{ mm}^2$ was printed in 3 min with the nib speed of 2.5 mm s^{-1} (supplementary

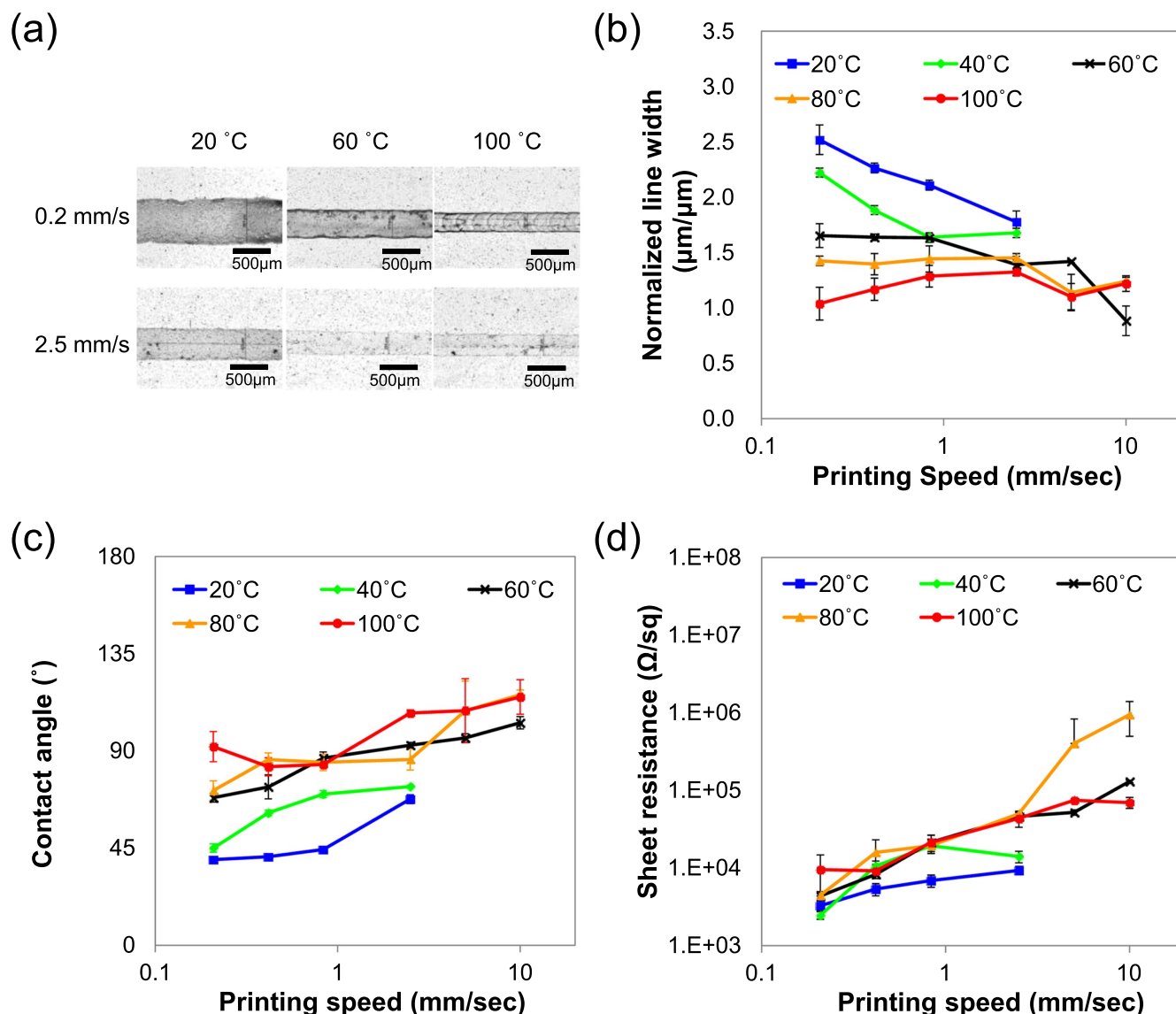


Figure 4. Line printing (a) printed lines at 0.2 and 2.5 mm s⁻¹ under the substrate temperature of 20 °C–100 °C. (b) Normalized line widths at a temperature of 20 °C–100 °C with a printing speed of 0.2–10 mm. (c) Advancing contact angle ($\theta_{B,a}$) at various printing speeds. (d) Sheet resistance according to print temperature and speed.

material; movie file). When the printing lines were overlapped at $T = 20$ °C, the nanoink was smudged across the printed lines. The optical transparency of the film increased from 67% to 89% as the temperature increased (figure 5(a) and supplementary information; figure S7). For print temperatures >60 °C, SWCNT clusters were observed; during printing, the SWCNTs were aggregated at the stopper, leaving clusters on the substrate. The sheet resistance increased from 2.5 to 62.4 kΩ/sq as the temperature increased (figure 5(b)), due to the larger bridge contact angles and thus the reduced ink flow rate. The sheet resistance was the highest at 80 °C and reduced at 100 °C, consistent with line printing observations.

4.2. Doping effect

The section aims to characterize SWCNT lines doped with PEI. In comparison to PEI/SWCNT printing, SWCNT/PEI printing shows consistent resistance.

For SWCNT/PEI lines, the current decreased as the number of PEI deposition layers increased (figure 6(a)) based on I - V characterization (inset figure of figure 6(a)). For the repeated tests ($N = 3$), the error bars for the electric current showed ± 9.5 , ± 6.8 , ± 5.3 , $\pm 1.6\%$ for 0, 1, 2, and 3 depositions. With more depositions, the error bars were reduced because semiconducting SWCNTs with various chirality reached current saturation by the doping. Considering the initial p-type SWCNTs, the current decreased due to the PEI' amine group doping. For measurement over 1000 s, the resistance change was only $\pm 0.09\%$, showing layer stability (figure 6(b)).

For PEI/SWCNT lines, the doping effect was not as reproducible as the SWCNT/PEI lines (figure 6(c)): the SWCNTs might not be fully covered with PEI. The resistance could increase or decrease for forward and backward printing directions (figure 6(d)). The large change in the resistance appeared to be related to the physisorption and reaction with air.

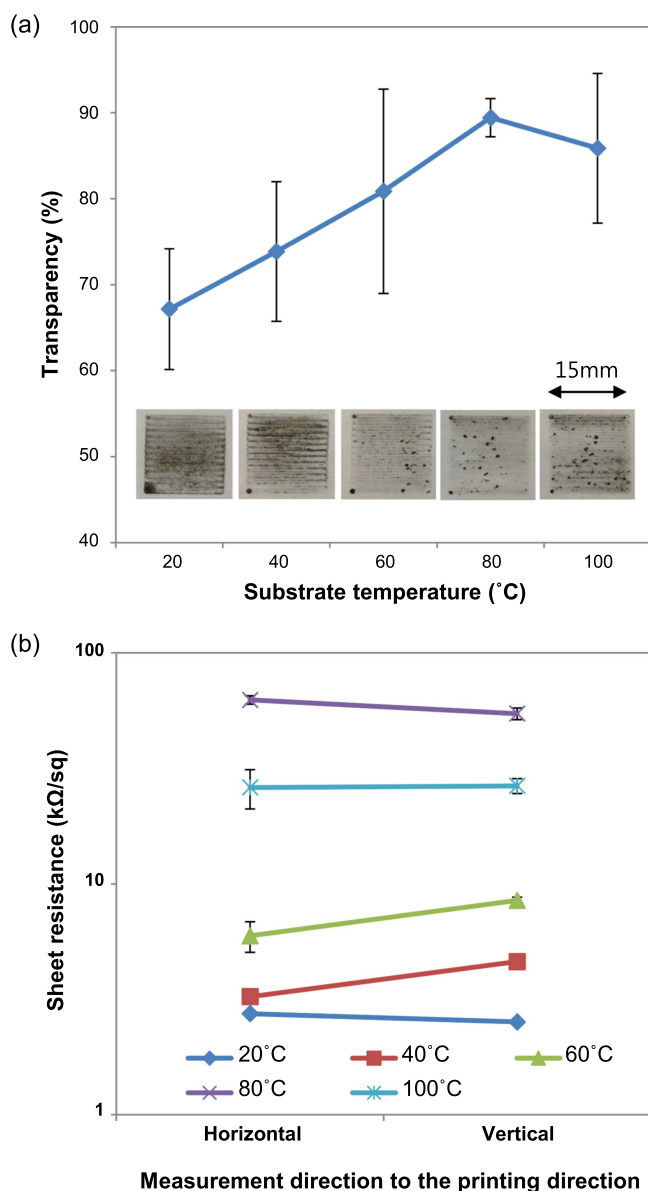


Figure 5. (a) Transparency of printed films at temperatures of 20 °C–100 °C. Printing speed: 1 mm s⁻¹ (b) Sheet resistance in the printing and its vertical directions.

When the SWCNT device was placed in a vacuum chamber (125 mmHg), the resistance was constant because of low oxygen environment (figure 6(d)). Figures 6(c) and (d) suggest that the I – V nonlinearity resulted from the continuous change of PEI/SWCNT resistance due to the interaction with air.

4.3. Sensor evaluation

4.3.1. Gas sensor. The section aims to characterize a SWCNT-gas sensor. When SWCNTs were doped with PEI and Nafion, the sensor showed selectivity due to electrostatic interaction. PEI doped SWCNTs could selectively detect NO₂ while Nafion-doped SWCNTs could selectively detect NH₃.

The non-contact printing technique was used for fabrication of a gas sensor. To achieve uniform resistance, PEI was

coated on a SWCNT line. The SWCNT sensor doped with PEI showed a response to NO₂ gas (figure 7(a)). Nafion-doped SWCNTs showed a negligible change when exposed to NO₂ gas but had a significant response to ammonia (supplementary information; figure S9). The specificity of the SWCNT-sensors to NO₂ and ammonia gases were consistent with the previous report [26]. However, the response trends were opposite from the reported trends using n-type SWCNTs. The doping can change the sensitivity and selectivity of a SWCNT sensor [27]. For example, a positively charged dopant makes a SWCNT sensor sensitive to negatively charged gas molecules but insensitive to positively charged gas molecules. Without doping, a SWCNT sensor is also sensitive to gas molecules regardless of the electric charge of gas molecules (supplementary information; figure S9). Note that our results were from the SWCNTs printed on a PET film while the previous report used semiconducting SWCNTs grown on the micromachined electrodes.

4.3.2. pH sensor. The section aims to characterize a SWCNT-pH sensor. The sensitivity showed 61 mV/pH.

For pH measurement, solution drops of pH = 4, 7, and 10 were sequentially interrogated using the fabricated sensor. The potential decreased as pH changed from pH 4 to 10. Note that the actual potential needed to be subtracted from the measured value because a 1.61 V bias potential was added to the circuit. The average slope was 61 mV/pH at 100 s was consistent with the theoretical Nernstian slope. At the low pH between 4 and 7, the sensor showed more stable voltage potential, which agreed with the previous report [28]. In comparison to the previous report, the fabrication method presented in this paper is more cost-effective.

4.4. Discussion

Due to the non-contact nature of the bridge-induced printing, the SWCNTs could be deposited without damaging substrate surface. The contact mode printing with the same capillary pen resulted in scratch marks on the substrate. The print line width was determined by the pen's outside diameter and the contact angle. For the examined ink formulation and substrate type, the contact angle was a function of substrate temperature and print speed. The smallest line width was obtained at $\theta_{B-a} \sim 90^\circ$ and was equal to the outside diameter of a pen nib. According to our characterization, PEI solution with known surface tension coefficient and viscosity showed that the line width was determined by solution viscosity at low speed (supplementary information; figure S8). As the speed increased, the line width was determined by the contact angle and Capillary number. Since the flow rate decreased with the higher contact angle, the resulting sheet resistance increased at the lower flow rate. For example, using the smallest ink drop radius (110 μm) and water surface tension coefficient (0.073 N/m), the pressure difference (ΔP) at the substrate surface could range from 0 to 1.3 kPa. At room temperature, ΔP was close to 0 kPa due to the spreading of nanoink, which increased to 1.3 kPa at $\theta_{B-a} = 90^\circ$. Considering that the working principle yields very low pressure gradient

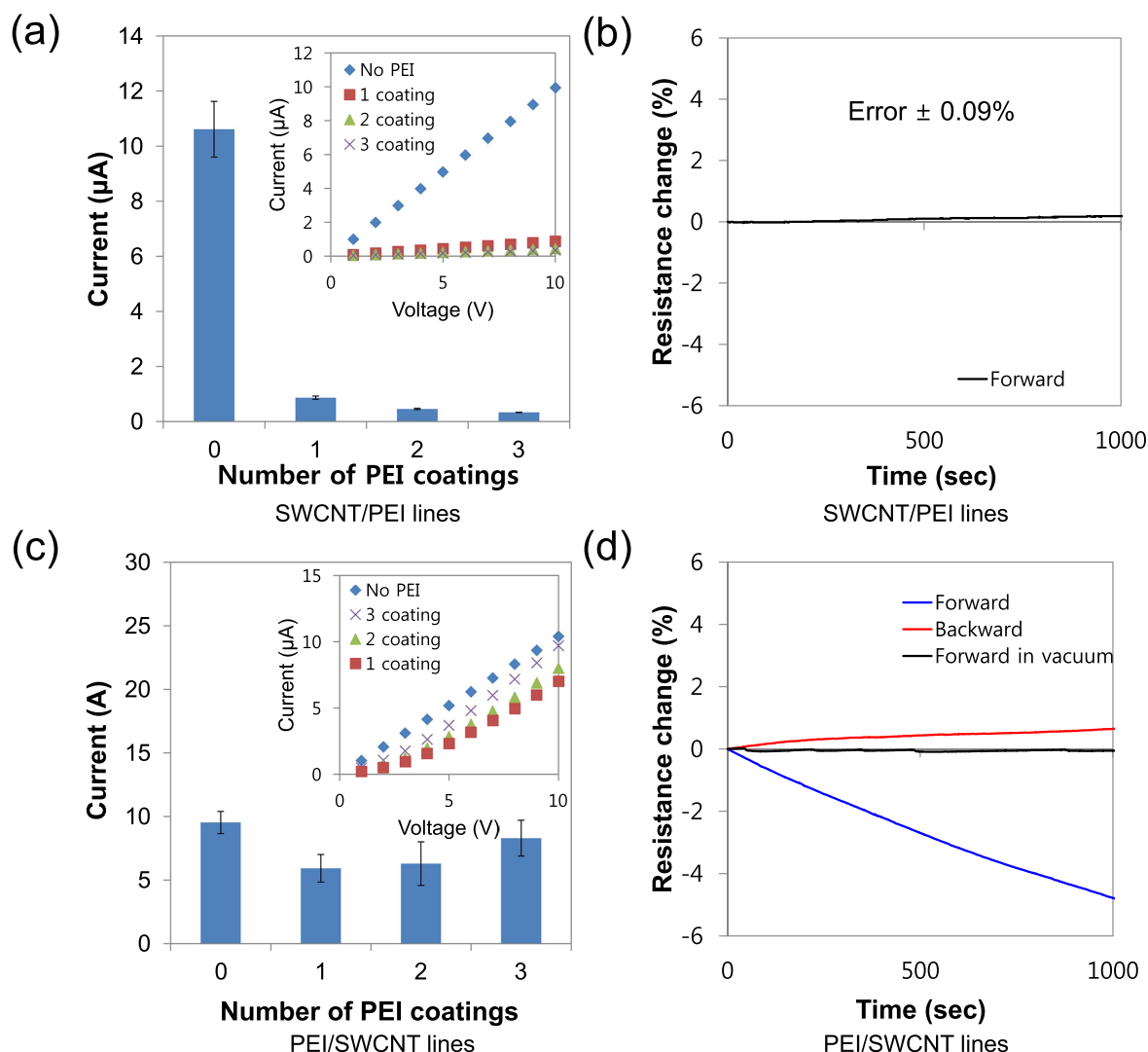


Figure 6. Resistance change for SWCNT/PEI and PEI/SWCNT lines (a) current change at 10 V for 1, 2, and 3-PEI depositions. (b) Resistance change for a SWCNT/PEI line. (c) I - V characteristics of PEI/SWCNT lines for 1, 2, and 3-PEI depositions. (d) The resistance change of a SWCNT device for forward and backward printing directions in the air and forward direction in vacuum (125 mmHg).

in the system, the non-contact nanoink bridge-induced printing method can be beneficial for printing water-based molecular ink.

5. Conclusions

In summary, we developed a non-contact capillary pen printing method for fabrication of SWCNT chemical sensors. Using a custom printer, the patterns of a dot, a line, and a film were printed and characterized in the contexts of morphology, electrical properties, and optical transparency. During the printing process, the contact angle was measured and related to the substrate temperatures and printing speeds. For dot printing, a coffee ring effect was clearly shown for a high temperature substrate due to the rapid evaporation and the pinning effect in the ink bridge. The contact angle gradually

decreased at room temperature, which formed a relatively uniform height of an SWCNT dot. For line printing, the advancing contact increased as the substrate temperature and the print speed increased. For these conditions, the higher pressure at the substrate reduced the ink flow rate increasing the sheet resistance and the optical transparency of the SWCNT line. For print uniformity, optimal printing temperatures were in the 60°C – 80°C range. A film could be printed to obtain an average sheet resistance of $7.2\text{ k}\Omega/\text{sq}$ by a single printing at 60°C . To obtain consistent high-quality prints, the advancing contact angle needs to be monitored. The nanoink bridge-induced printing allows for printing complex sensor geometries without damage of previous layers. Consistent results have been obtained in the application as a target selective gas sensor and a pH sensor. The non-contact printing approach facilitates printing of large array sensors at low cost for wearable and film-type platforms.

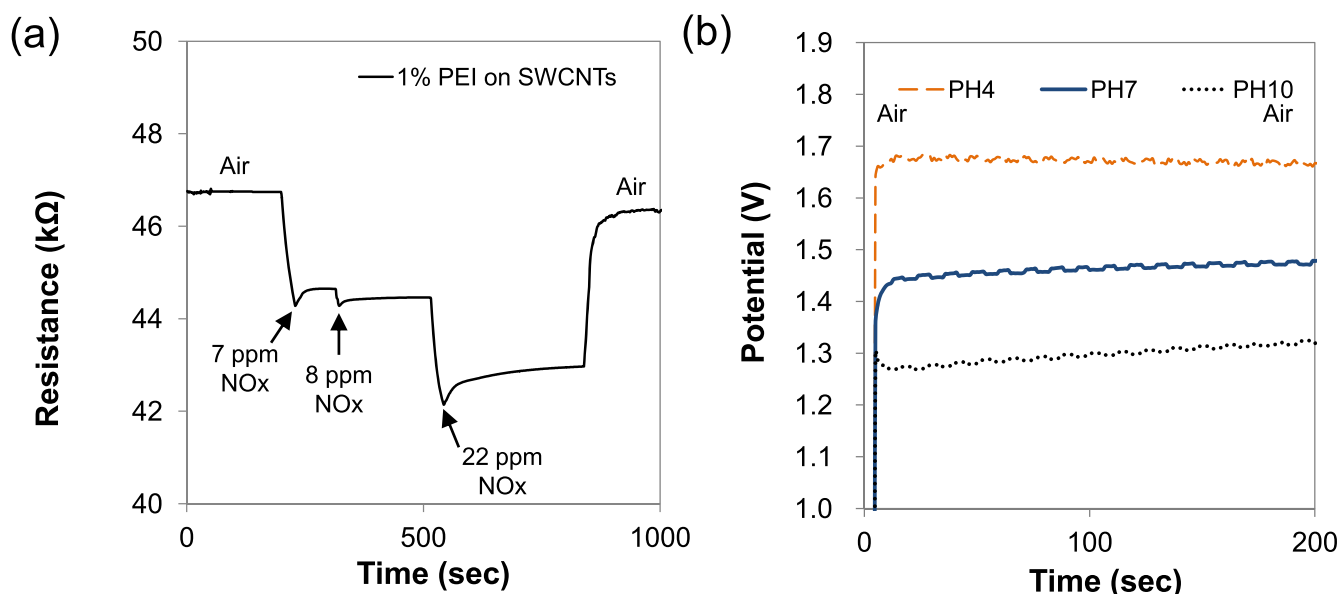


Figure 7. Gas and pH response test (a) change of PEI doped SWCNT resistance for NO₂ gas; PEI is doped on a SWCNT line. (b) The voltage measured for a pH sensor using standard solutions of pH 4, 7, and 10. Note that the voltage is shifted by using a 1.61 V AA battery.

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Seong-Joong Kahng

Wearable Carbon Nanotube Sensors: Fabrication and Applications for Bio/Chemical Sensors

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Abstract

**Wearable Carbon Nanotube Sensors:
Fabrication and Applications for Bio/Chemical Sensors**

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Chair of the Supervisory Committee:
Associate Professor Jae-Hyun Chung
Department of Mechanical Engineering

With the advancement of micro/nanotechnology, wearable device technology is rapidly changing our lifestyle. Wearable devices armed with high-performance sensors potentially offer real-time health monitoring of human body conditions and produce massive database correlating physiological parameters with diseases, health, and behavior. However, most wearable devices do not offer wearer's comfort because the manufacturing methods are based on a stiff silicon substrate. Among nanomaterials, carbon nanotubes (CNTs) are emerging as electronic or sensing materials on a flexible substrate. For such devices, CNTs need to be patterned on a flexible substrate like polyethylene terephthalate (PET) film. Inkjet printing is one of the major patterning methods, but the pattern is discrete because of droplet-based printing. Also, inkjet

printing is limited by the ink properties, including surface tension and viscosity. Fountain pens can be used to print continuous lines but potentially damage the substrate. The contact printing methods may not be suitable to print multiple functional layers because the pen nib induces damage to existing layers.

In the dissertation, nano ink bridge-induced capillary pen printing is proposed as a novel method for continuous line printing of carbon nanotubes. Firstly, the control parameters of the noncontact capillary method are studied in terms of line width, edge roughness, and sheet resistance for uniform printing. Nanoink liquid bridge forms between the tip of the stylographic pen and substrate by capillary action. The printed pattern is characterized in the contexts of nano ink bridge formation between pen nib and substrate. Ink properties, printing temperature, printing speed, and contact angles are studied to find optimal printing conditions. The nano ink bridge-induced printing allows multiples layers of nanomaterials without damaging existing layers. This printing method facilitates the fabrication of low-cost wearable sensors on a flexible substrate. As printing applications, gas and pH sensors are demonstrated using the carbon nanotube pattern and chemical doping by the capillary pen printing method.

For biosensor fabrication, a point-of-care (POC) platform for tuberculosis screening is presented using a carbon nanotube film. Tuberculosis, caused by *Mycobacterium tuberculosis* (MTB), is one of the serious infectious diseases worldwide. Various methods are available for TB diagnoses, such as a Ziehl-Neelsen (ZN) method for microscopic detection, immunoassays for antigen detection, and polymerase chain reaction (PCR) for DNA or RNA detection. For a highly sensitive and specific screening tool, nanomaterials have been persistently investigated for infectious disease diagnosis. Resistive single-walled carbon nanotube (SWCNT) sensors have shown potential for rapid TB screening. However, hydrogen bonding on SWCNTs interferes the

resistance change due to target binding. In this dissertation, a resistive SWCNT biosensor is fabricated on a flexible film (PET) for low-cost TB screening. Silver electrodes are stamped as probing electrodes for SWCNTs. The sensing mechanism of SWCNTs, coupled with silver electrodes, is investigated in conjunction with hydrogen desorption. The sensitivity and specificity are characterized by MTB and surface antigen (MPT64) in physiological buffer. Subsequently, the sensor is characterized by tongue swab samples spiked MTB and MPT64. Simple resistive measurement is conducted before and after immunocomplex formation for detecting targets. The presented biosensor will offer a stepping stone for an inexpensive and versatile POC platform for rapid TB screening.

In summary, the critical challenges for SWCNT-based wearable sensors are addressed in terms of scalable fabrication and hydrogen bonding. The printing physics is investigated for nano ink-bridge induced printing of SWCNTs. A rapid TB screening sensor is developed with the investigation of hydrogen adsorption and desorption effects on SWCNTs. A thin, flexible sensing platform will facilitate the scalable fabrication of bio- and chemical sensors with low cost.

TABLE OF CONTENTS

List of Figures	iii
List of Tables	viii
Chapter 1. Wearable sensors.....	1
1.1 Introduction.....	1
1.2 Wearable sensors	4
1.3 Non-contact printing methods.....	13
1.4 Challenges.....	23
1.5 Objectives	24
Chapter 2. Nanoink Bridge-induced Capillary Pen Printing	26
2.1 Introduction.....	26
2.2 Objectives	27
2.3 Nanoink bridge-induced printing.....	27
2.4 Experimental methods	30
2.4.1 Characterization of the printing method	30
2.4.2 Doping effect	34
2.5 Results.....	34
2.5.1 Characterization of the printing method	34
2.5.2 Doping effect	43
2.6 Discussion.....	45
2.7 Conclusion	47

Chapter 3. Fabrication and characterization of chemical sensors.....	49
3.1 Introduction.....	49
3.2 Objectives	52
3.3 Sensor fabrication	52
3.4 Experimental results.....	56
3.5 Discussion	62
3.6 Conclusions.....	63
Chapter 4. a flexible immuno-sensing platform using single-walled carbon nanotubes (SWCNT)	64
4.1 Introduction.....	64
4.2 Objectives	67
4.3 Experimental method	68
4.3.1 Sensor fabrication	68
4.3.2 Antibody preparation	72
4.3.3 Sensor characterization	74
4.3.4 Sensitivity and specificity tests.....	75
4.3.5 Test using tongue swab samples	76
4.4 Experimental Results	77
4.5 Conclusions.....	88
References.....	89

LIST OF FIGURES

Figure 1.1. [1]. The estimated market of wearable devices between 2016 and 2020 (CCS Insight in 2016)	1
Figure 1.2. [8]. The scheme of the wireless network for wearable sensors	2
Figure 1.3. [11]. Battery-free small sized flexible sensors used for full-body monitoring. (A) Mapping data of body temperature and pressure through a wireless network. (B) Temperature and pressure sensor layout with NFC microchip (C) The device structure.	4
Figure 1.4. [15] (a) Comparison of temperature response between the flat sensor and bent sensors of different bending curvature (b) Resistance deviation of the temperature sensor under different bending radius. (c) Longtime measurement of the temperature sensor in the atmosphere and water. (d) Resistance deviation while stretching the arm and unclenching the fist.....	5
Figure 1.5. [16]. Epidermal temperature sensors (a) Images of a 4×4 the temperature coefficient of resistance (TCR) sensor array integrated on an elastomeric substrate (b) Infrared image of a similar device attached on human skin (left) and map of temperature (right), where each pixel represents the data of each sensor in the array. (c) Optical images of an 8×8 Si nano-membrane diode sensor array integrated on a thin elastomeric substrate (d) Optical image of a similar device mounted on a heater (left) and measured distribution of temperature (right).	6
Figure 1.6. [17] (a) image of wearable and wireless heart rate sensor and (b) structure of the pressure sensor using ZnO/PVDF hybrid film between rGO electrodes. (c) The I–V curves of the pressure sensor that rectifying the behavior of Schottky contact under compressive strain. The insets show a log I vs. V plot and schematic illustration of forward bias when pressure is applied.....	7
Figure 1.7. [18]. Blood pressure sensor using spherical bump PVDF HFP/PEDOT 3D NF mats. (a) Schematic view of the sensor matrix (left) and single-unit sensor systems (right). (b) Images of a PVDF-HFP/PEDOT array on an inkjet-printed electrode PEDOT: PSS/ PET	

film, (c) circuit board for pressure mapping (top) and a Bluetooth board (bottom). (d) Blood pressure mapping using a PVDF-HFP/PEDOT array sensor.	8
Figure 1.8. [21]. (a) The fabrication process of a vertically aligned SWCNTs strain sensor. (b) A bandage strain sensor fixed to a neck	9
Figure 1.9. [21] (a) Model of the strain sensor. R_1 , R_2 , and R_c are the resistances of the island, gap, and stretched elongation bridge, respectively. (b) Average island width (blue) and gap (red) versus strain for cycling following the conditioning step.	10
Figure 1.10. [29]. Illustrate a wearable sweat sensor. (a) a spiral-patterned microfluidic channel, Au electrodes for sweat rate sensing, an insulation layer, and Na^+ sensing electrodes. (b) Structure of the microfluidic device. The aligned Au electrodes with the microfluidic channel. The Na^+ sensor at the sweat collection reservoir. (c) The sweat sensor on human skin. The collected data from the sensor can be transferred to a cellphone through a wireless network.	12
Figure 1.11. [32]. Patterned CNTs grid by inkjet printing and EL devices.	13
Figure 1.12. [42]. Structure of the micromachined droplet ejector.....	14
Figure 1.13. [35]. (a) Schematic image of pyroelectrodynamic printing using the LN. (c) Various patterns by pyroelectrodynamic printing, such as separate droplets, straight and curved lines.	16
Figure 1.14. [36]. Schematic of the E-jet printing set-up (up) and printed patterns using constant voltage jetting (down left) and pulsed voltage jetting (down right).	17
Figure 1.15. [37]. (a) Schematic view of the SAW (b) Relation between the surface acceleration magnitude and the drop size and behavior of the drop.	19
Figure 1.16. [38]. (a) Schematic of the Dielectrophoretic (DEP) printing setup. (b)–(d) The dispensing mechanism of the Dielectrophoretic (DEP) printing (e) demonstrates the electric field distribution of the nozzle. (f) Scanning electron microscopy image of glass capillary (g) Scanning electron microscopy image of corresponding Au-dots by DEP printing. .	20
Figure 1.17. [39]. Illustration of dip-pen nanolithography (DPN) writing.	21
Figure 2.1. Nanoink bridge-induced capillary printing (a) Printing concept (b) Schematic of an XYZ plotter installed with a heating stage and a camera system. The top image shows a printing system, and the bottom is a photograph of the setup. (c) Nanoink-bridge induced	

printing using water ink on a PET film. The ink is released with pressing the stopper. Upon withdrawal by 100 μm , an ink bridge forms. The advancing contact angle increases as the pen moves from left to right. (d) W-pattern printed by SWCNT-ink at 80°C at 1.2 mm/sec. The top image shows a design.	29
Figure 2.2. Diameters of the capillary pens. The nominal diameters of the capillary pens are 100, 300, and 700 μm . The outer diameters of the pen nib are 225, 375, and 790 μm , respectively.	31
Figure 2.3. 4-point probe measurement setup. The sheet resistance is measured using a custom 4-point probe measurement system. The distance between electrodes is 2.5 mm....	33
Figure 2.4. Dot printing (a) Dots printed at surface temperature 20~100°C with holding time of 1, 5, and 10 s. Scale bar: 500 μm (b) Dot diameters normalized by an outer nib diameter (c) Contact angles according to holding time (d) Dot profile at 20, 60, and 100°C (1s holding time).	35
Figure 2.5. Line printing (a) Printed lines at 0.2 and 2.5 mm/s under the substrate temperature of 20~100 °C. (b) Normalized line widths at a temperature of 20~100 °C with a printing speed of 0.2~10mm. (c) Advancing contact angle (θ_{B_a}) at various printing speed. (d) Sheet resistance according to print temperature and speed.	37
Figure 2.6. Printed SWCNT lines at 20 and 100 °C. Top rows are SEM images, and bottom rows are optical microscope images for 20 and 100°C, respectively. The beach-mark pattern is observed at 100°C due to a stick and slip effect. The two lines at the edge of an SWCNT line at 100°C A form by a coffee ring effect.	39
Figure 2.7. Transparency for an SWCNT line according to various printing speed at 20°C.	40
Figure 2.8. (a) Transparency of printed films at temperatures of 20~100°C. Printing speed: 1 mm/s (b) Sheet resistance in the printing and its vertical directions.	42
Figure 2.9. Resistance change for SWCNT/PEI and PEI/SWCNT lines (a) Current change at 10 V for 1, 2, and 3-PEI depositions. (b) Resistance change for an SWCNT/PEI line. (c) I-V characteristics of PEI/SWCNT lines for 1, 2, and 3-PEI depositions. (d) The resistance change of an SWCNT device for forward and backward printing directions in the air and forward direction in a vacuum (125 mmHg).	44

Figure 2.10. Line width for various concentrations of PEI diluted in deionized water. ..	46
Figure 3.1. Fabrication steps (a) Images and cross-section of an SWCNT-gas sensor (b) Optical and SEM images and fabrication steps of an SWCNT-pH sensor.....	53
Figure 3.2. Silver electrode patterning. Sensor electrodes for a pH sensor are screen-printed to form silver electrodes on a PET film. The mask material for screen printing is PET film	55
Figure 3.3. Gas response test (a) Change of PEI-doped SWCNT resistance for NO _x gas, (b) Change of Nafion-doped SWCNT resistance for NO _x gas.	57
Figure 3.4. Change of Nafion-doped SWCNT resistance for ammonia gas. Nafion can differentiate between NO _x and Ammonia with high accuracy, showing specificity.	58
Figure 3.5. (a) Response of 0.1% Nafion-doped SWCNT, non-doped SWCNT and MQ-135(commercial) sensors for ammonia concentrations of 1, 20, 120 and 2,580 ppb. (b) The sensitivity of SWCNT sensors with 0.1% Nafion and 1% Nafion doping and without doping. Normalized resistance change vs. ammonia concentrations.	59
Figure 3.6. Fabricated pH sensor	60
Figure 3.7. (a) Voltage measured for a pH sensor using standard solutions of pH 4, 7, and 10. Note that the voltage is shifted by using a 1.61 V-AA battery. (b) Stability of a pH sensor for 20,000 seconds	61
Figure 4.1. (a) An SWCNT-based sensor on a flexible PET film. (b) Cross section of a resistive SWCNT immunosensor.	69
Figure 4.2. The fabrication process of an SWCNT-based immunosensor (a) Spin coating of SWCNTs on a PET film (b) Spin coating of PEI (c) Stamping of silver electrodes. (d) Antibody immobilization (e) Photo and optical microscope images of an SWCNT immunosensor.	71
Figure 4.3. (a) Optical density showing the binding of MPT64 antibodies to MTB (10 ⁶ CFU/mL) and BCG (10 ⁶ CFU/mL) at 28 µg/mL. (b) Optical density showing the binding of MPT64 antibodies to MPT 64 in comparison to control.....	73
Figure 4.4. Preparation protocol of tongue swab samples and resistive detection procedure.	77

Figure 4.5. (a) Normalized resistance change before and after antibody immobilization on SWCNT sensors (N=4) (a) Normalized resistance change of an SWCNT immunosensor at 25 and 35 °C after antibody immobilization.....	78
Figure 4.6. (a) The resistance change of an SWCNT immunosensor at 25 and 35 °C after antibody immobilization. (b) Normalized resistance change of a SWCNT sensor for control and MTB (10^6 CFU/mL) in PBS (N = 4). The sensor is tested after 5, 20, 40, and 120 min incubation at 25 °C.....	80
Figure 4.7. Resistances of 0.1% PEI coated SWCNTs and antibody-coated SWCNTs. The resistance is measured after 2 hours at 35 °C.	81
Figure 4.8. (a) Sensitivity test for MTB in PBS. (b) Sensitivity test for MPT64 in PBS.	83
Figure 4.9. Specificity test results for MTB (10^2 CFU/mL), S. Epi (10^3 CFU/mL), M. Avium (10^3 CFU/mL), and M. BCG (10^3 CFU/mL).	84
Figure 4.10. (a) Fluorescence microscope images for an MTB colony (10^6 CFU/mL). (b) Control samples. The grey area is the SWCNTs on a PET film. The black area is a silver electrode. (c) and (d) SEM images of MTB cells (10^6 CFU/mL).	85
Figure 4.11. Detection limit tests for MTB and MPT 64 (a) MTB spiked in tongue swab samples, (b) MPT64 antigen spiked in tongue swab samples.	86
Figure 4.12. (a) Bending test using 3 mm silicone bar (b) Resistance change for the 1 st bending and the 1 st recovery (N=6).	87

LIST OF TABLES

Table 1. [42]. Characteristics of printing methods.....	22
Table 2. Resistive single-walled carbon nanotubes for detection of bioanalytes.....	66

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After one year at the University of Washington, I was trying to go back home because I couldn't find an advisor and a research topic. Blindly, I sent an email to Professor Jae-Hyun Chung to ask him whether I could get a chance to join in his lab. That's the start of my graduate study. I think that I am able to be here, thanks to my advisor. I also would like to thank Professor Junlan Wang, Professor Igor Novosselov, and Professor Youngjun Choe. They spared valuable time and supported me with good feedback.

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DEDICATION

To my parents

Chapter 1. WEARABLE SENSORS

1.1 INTRODUCTION

With the pursuit of low-cost and portable smart technology, the form factor of sensors significantly decreases. The devices become cheaper and require minimal energy consumption. Wearable sensors play a critical role to obtain real-time information about human health and behavior in smart devices. According to the CCS Insight report in 2016 [1], the market of wearable technology will grow \$34.2 billion in 2020. The total number of smart wearable devices will be 411 million units by 2020 (**Figure 1.1**)[1]. The products can be applied for health monitoring, disease diagnosis, and disease treatment. Fitness, activity & sports trackers will occupy about 76% among the segments.

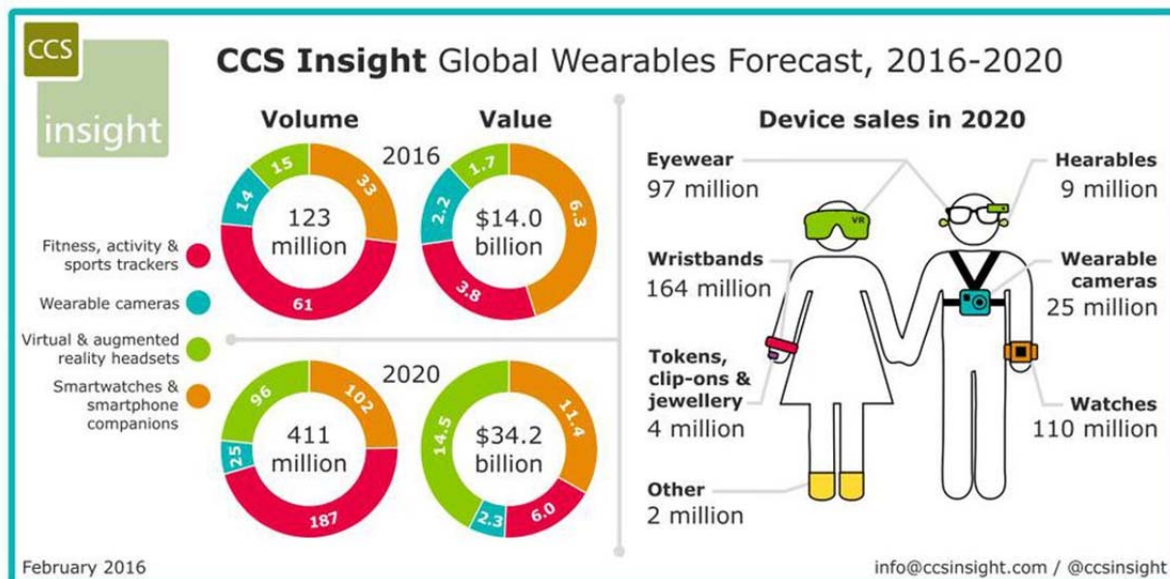


Figure 1.1. [1]. The estimated market of wearable devices between 2016 and 2020 (CCS Insight in 2016)

One of the promising fields in wearable devices is human behavior monitoring for healthcare. Physiological parameters, such as body temperature, heart rate, blood pressure, can be continuously monitored in daily life [2-4]. For example, the glucose level has been measured for diabetic patients with smart contact lenses in the eye [5, 6]. Smart contact lens provides painless and continuous glucose level monitoring while a conventional glucose meter is painful and discrete because human blood needs to be collected from the finger with an injection needle [7]. A normal pulsometer is embedded into a watch for continuous monitoring of heartbeats. These wearable monitoring devices are connected to a wireless network through a smartwatch to transfer the body information to a remote health care system for necessary treatment (**Figure 1.2**)[8].

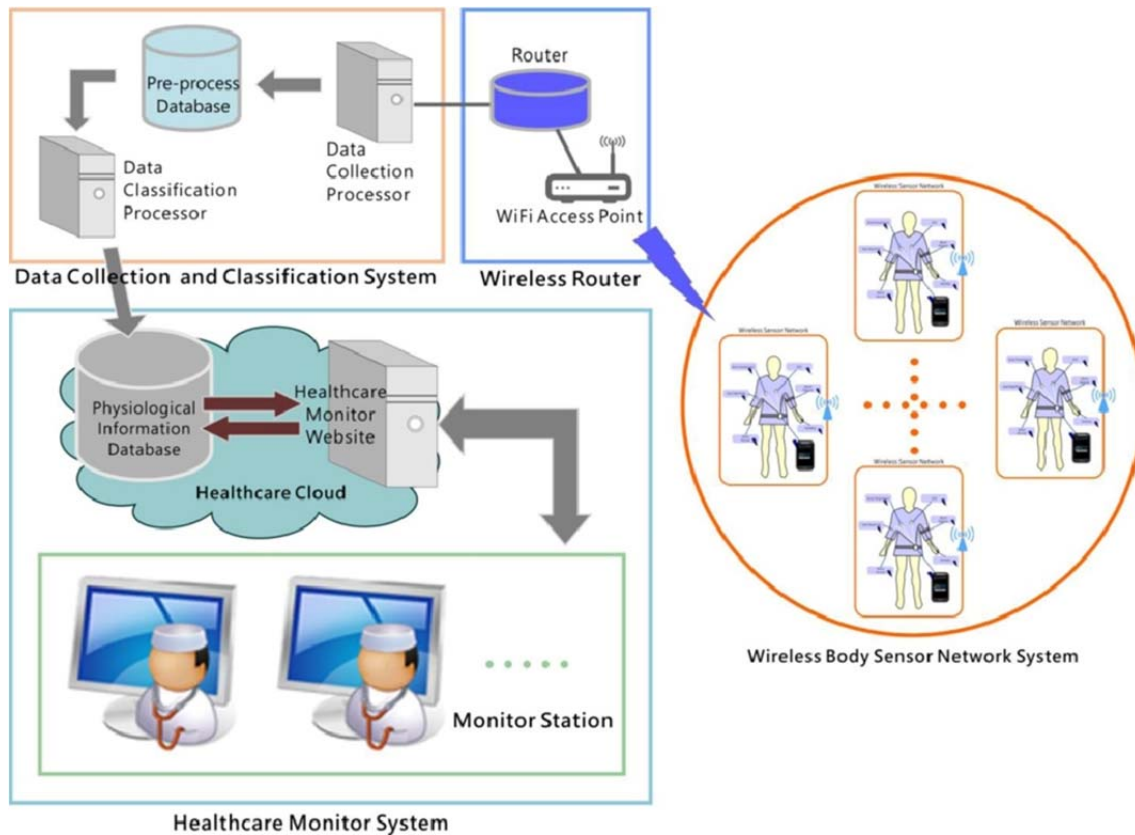


Figure 1.2. [8]. The scheme of the wireless network for wearable sensors

A small form factor is critical for portability and wearing comfort in wearable sensors [9]. Considering the major requirement for wearable sensors as flexibility, the substrate is limited to thin-film materials, such as paper, plastic, polymer, rubber. Low power consumption is an important factor for long-term operation and wireless data transfer [10]. To date, silicon-based semiconductor sensors have been used for smart devices because of the integration capability of various sensors into one chip. However, it is not flexible for curved human body profile. Although there are different types of flexible sensors, it is challenging to integrate the various sensors on a single substrate because each sensor shows different material and electrical characteristics. Sensor integration on a flexible substrate is a key step to multiplex various physiological parameters.

Personalized treatment for each is one of the growing fields in health care. Vital signs, such as heart rate, blood pressure, body temperature, and respiratory rate, are the basic physiological parameters of continuous human activity monitoring. For wearable applications of the sensors to the human body, sensors need to be soft, thin, and flexible. For whole body monitoring, a sensor array is needed to map whole human body conditions. The location of previous sensors has been limited to a few critical areas in the human body, such as arm, chest, and head. Wireless power could be used to operate the sensors and measure the parameters. Near field communication (NFC) technology with small size flexible patches was introduced as one of promising human body monitoring methods because of the simple wireless power transfer capability (**Figure 1.3**)[11]. This technology could be applied to in-hospital treatment, rehabilitation, and physical training like fitness [12]. Distributed pressure sensors on the whole

body can provide important information to prevent decubitus ulcers [13]. All the data on the whole body could be collected to a central control system through the wireless network [11].

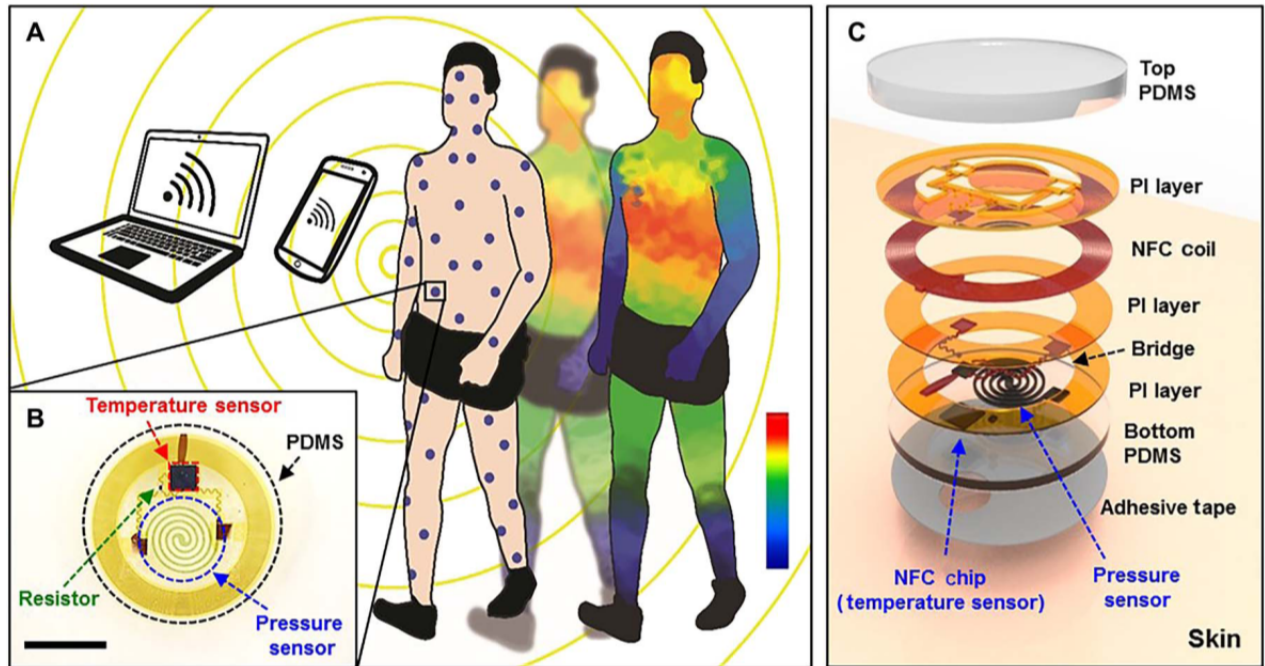


Figure 1.3. [11]. Battery-free small sized flexible sensors used for full-body monitoring. (A) Mapping data of body temperature and pressure through a wireless network. (B) Temperature and pressure sensor layout with NFC microchip (C) The device structure.

1.2 WEARABLE SENSORS

Temperature sensor: There are several test categories, such as electroencephalography (EEG) for electrical activity of the human brain, electrooculogram (EOG) for eye activity. One of the parameters is body temperature. Sleep disorder related to delayed sleep-wake phase, advanced sleep-wake phase, and jet lag can be diagnosed by mapping body temperature during sleep [13, 14]. A flexible temperature sensor based on graphite-filled polyethylene oxide (PEO) and polyvinylidene fluoride (PVDF) composites shows a high accuracy of 0.1°C between 25°C

and 42 °C [15]. The sensor shows consistent temperature performance under bending with different curvature and stretching on the skin surface (**Figure 1.4**). The sensing range from 25 °C to 42 °C for body temperature is ideal for medical diagnosis because the organs in the human body start to be damaged below 25 °C and over 42 °C. The sensing accuracy and physical stability of this sensor can be applied to a wearable device.

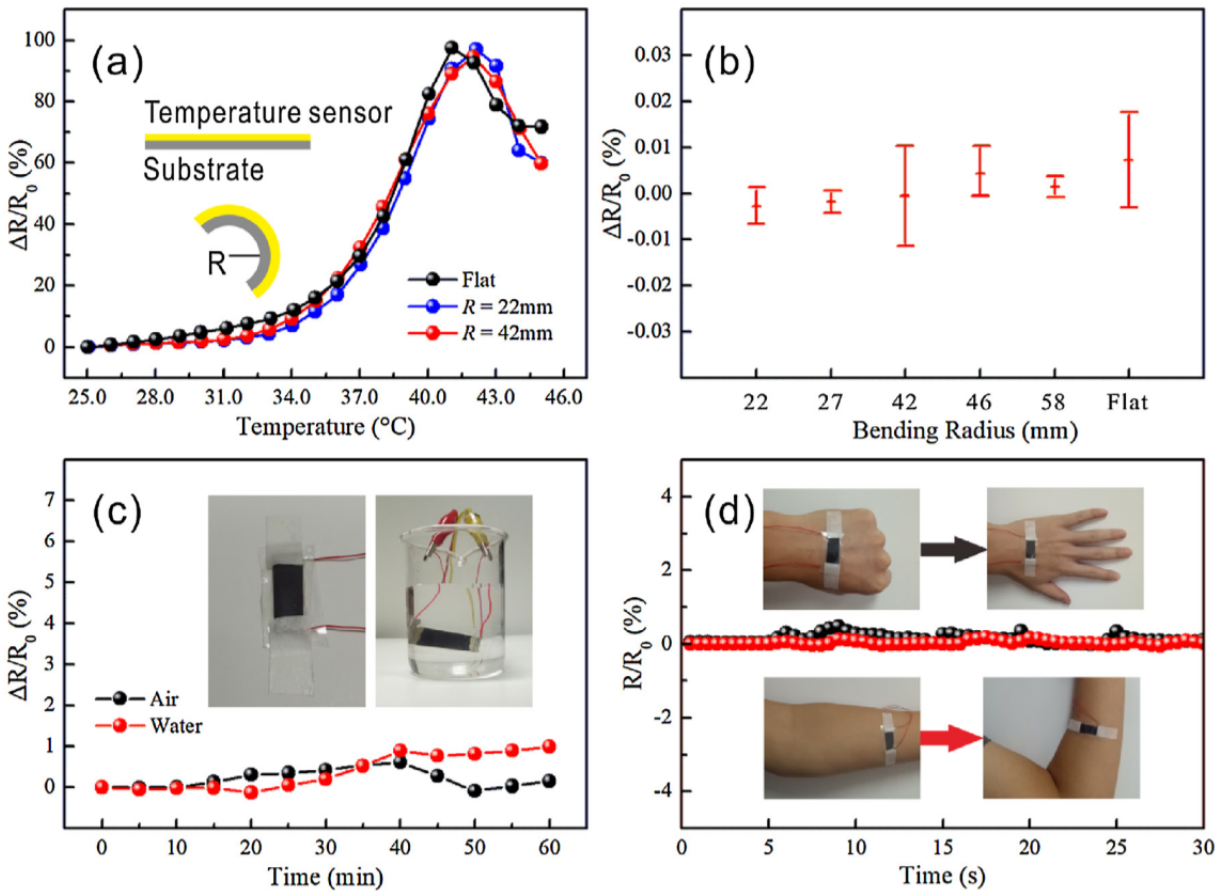


Figure 1.4. [15] (a) Comparison of temperature response between the flat sensor and bent sensors of different bending curvature (b) Resistance deviation of the temperature sensor under different bending radius. (c) Longtime measurement of the temperature sensor in the atmosphere and water. (d) Resistance deviation while stretching the arm and unclenching the fist.

The temperature coefficient of resistance (TCR) and PIN diode sensors are the resistant type of temperature sensor (**Figure 1.5**)[16]. These sensors are fabricated by semiconductor process on a flexible substrate, such as photolithography, etching, chemical vapor deposition, metal deposition. Due to the precise fabrication process, the sensor resolution is up to 1mm, and the sensitivity shows between 12 mK to 8 mK [16]. Although this sensor is wearable with high precision and ergonomic design, the fabrication is expensive and complicated.

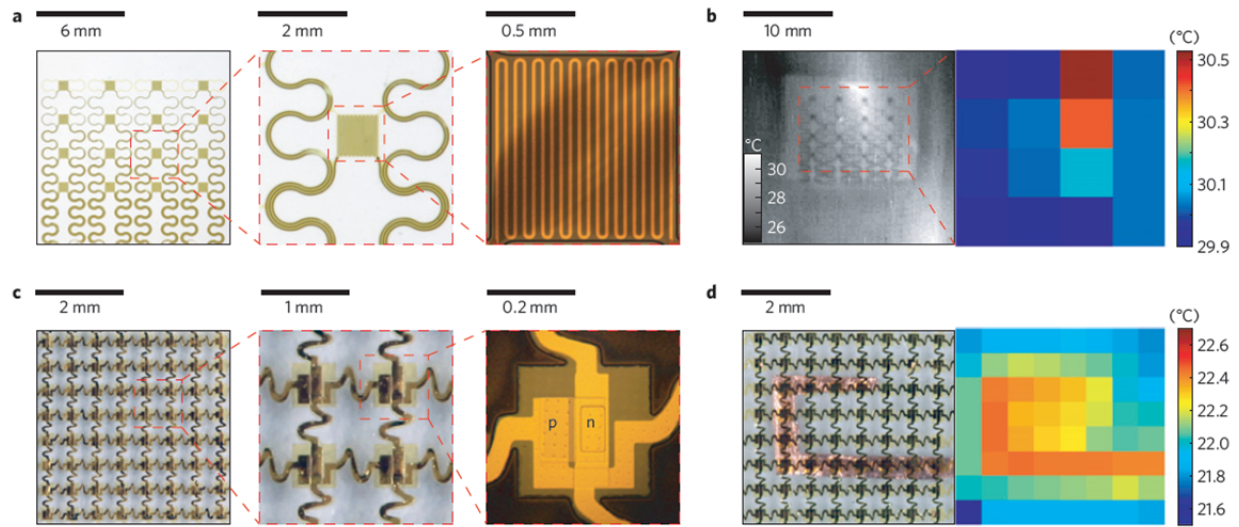


Figure 1.5. [16]. Epidermal temperature sensors (a) Images of a 4×4 the temperature coefficient of resistance (TCR) sensor array integrated on an elastomeric substrate (b) Infrared image of a similar device attached on human skin (left) and map of temperature (right), where each pixel represents the data of each sensor in the array. (c) Optical images of an 8×8 Si nano-membrane diode sensor array integrated on a thin elastomeric substrate (d) Optical image of a similar device mounted on a heater (left) and measured distribution of temperature (right).

Pressure sensor: Pressure sensors can be used for the heart rate monitoring through the pulse on the wrist or neck. A pressure sensor has been suggested on the zinc oxide (ZnO) nanoneedle/polyvinylidene difluoride (PVDF) hybrid film. The highly sensitive sensor could

detect down to 4 Pa [17]. The piezoelectric material produced an electrical potential by the deformation of the crystal structure due to an external force. The dielectric property increased a piezoelectric potential. The sensitivity of the PVDF-based sensor could be improved by the ZnO nanoneedle. This hybrid film sensor could be applied to heart rate monitoring in real time because of the rapid response and durability (**Figure 1.6**)[17].

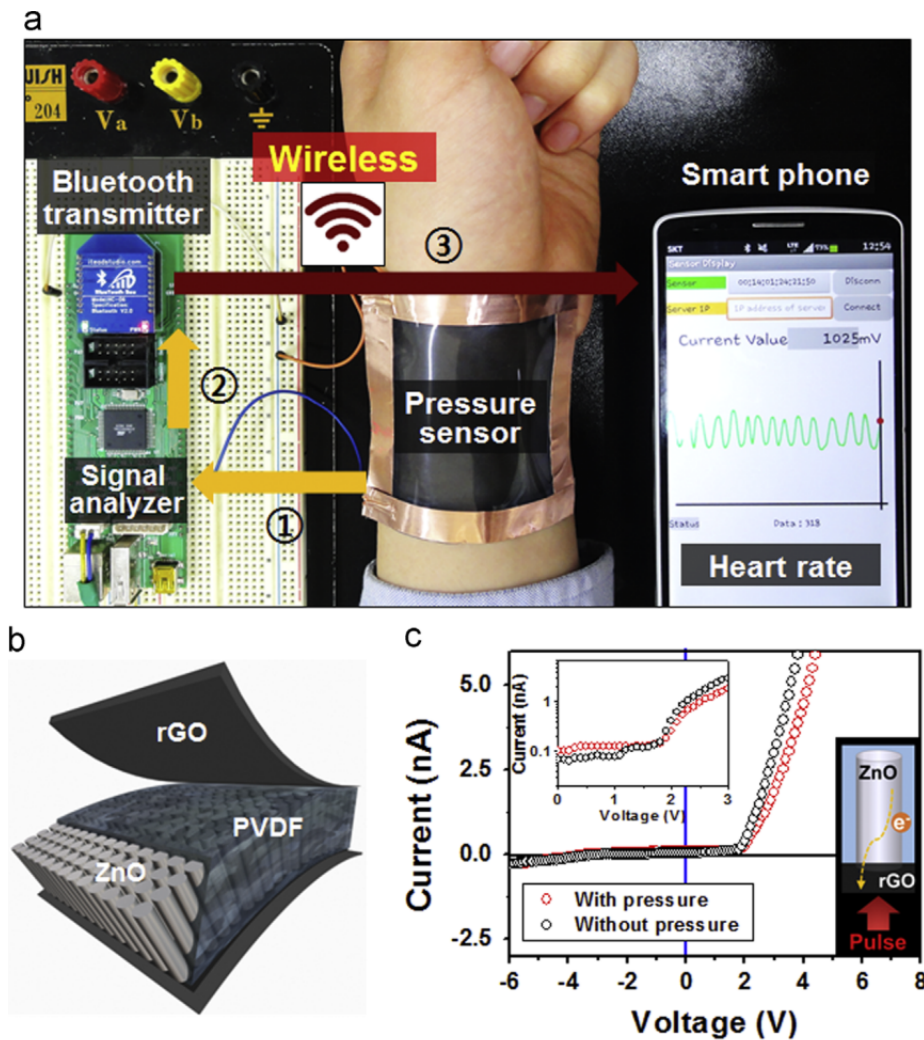


Figure 1.6. [17] (a) image of wearable and wireless heart rate sensor and (b) structure of the pressure sensor using ZnO/PVDF hybrid film between rGO electrodes. (c) The I–V curves of the pressure sensor that rectifying the behavior of Schottky contact under compressive strain. The insets show a log I vs. V plot and schematic illustration of forward bias when pressure is applied.

A piezoresistive sensor composed of polyvinylidene fluoride-co-hexafluoropropene (PVDF HFP) / poly(3,4-ethylenedioxythiophene) (PEDOT) were proposed as a blood pressure sensor (Figure 1.7) [18].

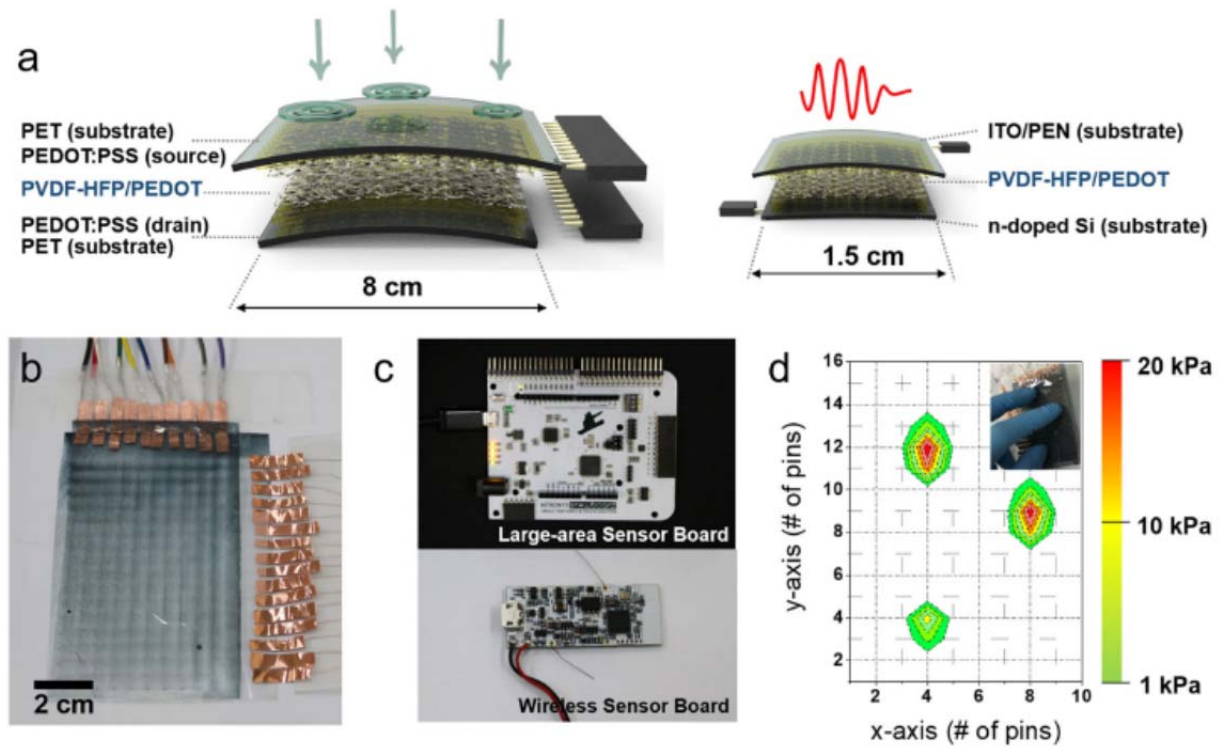


Figure 1.7. [18]. Blood pressure sensor using spherical bump PVDF HFP/PEDOT 3D NF mats. (a) Schematic view of the sensor matrix (left) and single-unit sensor systems (right). (b) Images of a PVDF-HFP/PEDOT array on an inkjet-printed electrode PEDOT: PSS/ PET film, (c) circuit board for pressure mapping (top) and a Bluetooth board (bottom). (d) Blood pressure mapping using a PVDF-HFP/PEDOT array sensor.

Strain sensor: Most piezoelectric strain sensors are highly sensitive, with small time constant and low power consumption. The main problems are poor flexibility, limited stretching capability, and low dynamic range. Piezo-resistive strain sensors consisting of CNTs have the

flexibility and good electrical property suitable to wearable sensors [19]. These can be utilized for human body movement by detecting the deformation of skin and joints. Damaged vocal cords, respiratory disorders, and angina can be diagnosed by monitoring abnormal behavior of muscle with a strain sensor. The CNT sensors can be applied to orthopedic applications to evaluate the inner spatial gap between bones and to determine the degree of change of spinal posture. Parkinson's disease can be diagnosed by detection of body movement of the arm, armpit, knee, waist, and spinal [20, 21] (**Figure 1.8**).

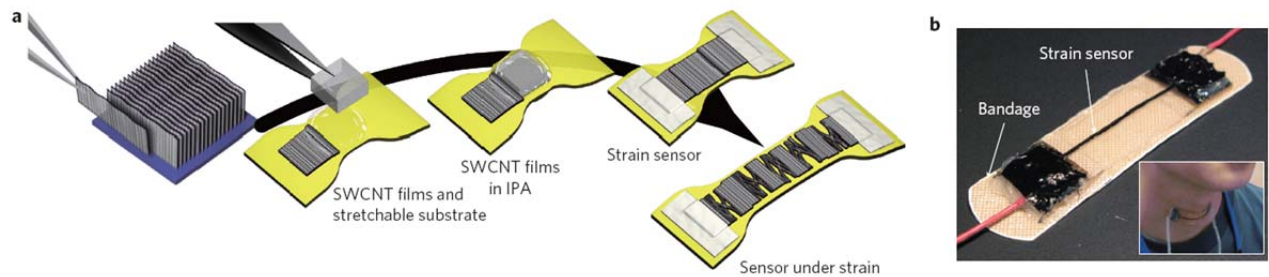


Figure 1.8. [21]. (a) The fabrication process of a vertically aligned SWCNTs strain sensor. (b) A bandage strain sensor fixed to a neck

For a strain sensor, a simple circuit model can be used (**Figure 1.9**). When a sensor is stretched, the resistance of R_1 is almost the same. With the bridge elongation, R_c shows linear behavior in the resistance value, while the resistance of R_2 increases exponentially with strain. The resistance change by strain is described as $R = 2R_1 + R_c$ [21].

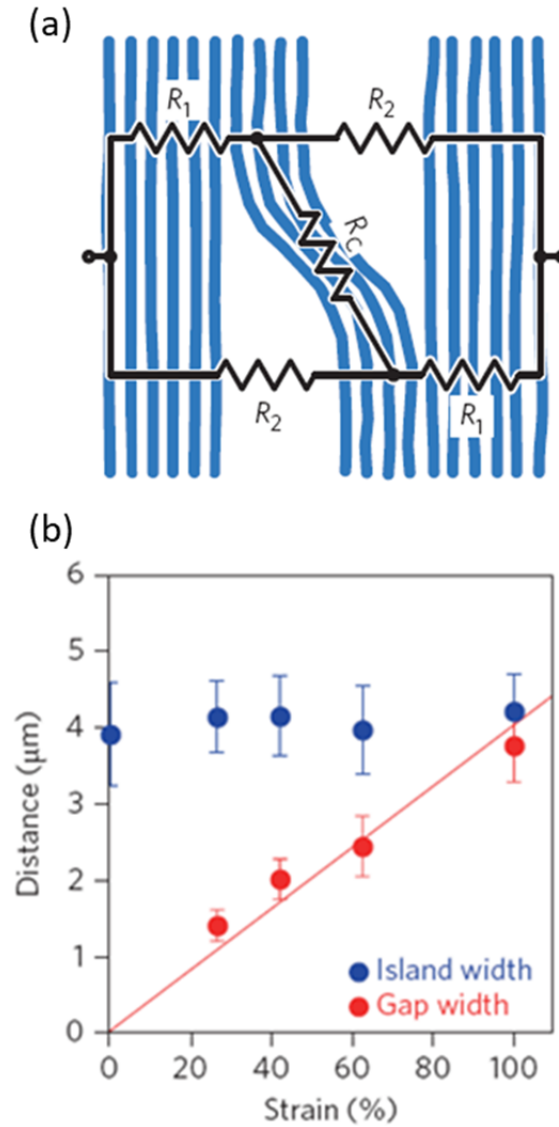


Figure 1.9. [21] (a) Model of the strain sensor. R_1 , R_2 , and R_c are the resistances of the island, gap, and stretched elongation bridge, respectively. (b) Average island width (blue) and gap (red) versus strain for cycling following the conditioning step.

Sweat sensor: A sweat sensor becomes one of the prime targets for health care because sweat contains critical data for health monitoring, disease diagnostic, and athletic performance evaluation [22]. Ions, metabolites, and hormones have been tried to apply for sweat analysis

using a sweat sensor [22]. Sweat sensors enable the measurement of the rate of sweat secretion and sweat sampling in real time. Sweating mechanism is complicated because the composition of ions and secretion rate are correlated [23]. Higher sweat rate increases the concentration of sodium and chloride ions. Dehydration during exercise makes sports ability worse [24]. In contrast, lactic acid and urea show higher proportion at a lower sweat rate [25]. Measuring the sweat rate has emerged as one of the key parameters to monitor physiological conditions.

Conventionally, sweat rate measurement has been tested in a specific test environmental with wired sweat patches [26]. One of the sweat rate sensors is an optical type that uses external light due to the lack of light in the sensing system [27]. Sweat sampling is complicated because sweat is prone to evaporate and contaminate with conventional methods. The sweat at the very beginning could be mixed with later discharged sweat, which could result in inaccurate data [28].

Micro-fluidic sampling can be a great alternative to these problems. An encapsulated microfluidics controls the samples without problems, such as evaporation condition, mixing of old and new samples, and contamination with another chemical [27]. There is a micro-fluidic sweat sensing patch with the chemical and electrical sensor inside the microfluidic channel for better analysis [29]. This sensor is composed of a flexible micro-fluidic channel and substrate for body attachment [29] (**Figure 1.10**). The microfluidic device detects ions in sweat and measures the sweat flow rate simultaneously to analyze the relationship between ion concentration and sweat secretion rate [29]. This system enables a real-time analysis for instantaneous feedback with the printed circuit board (PCB) that is integrated with signal processing and data transmission. With the integrated system, the existing problems could be eliminated due to the advantages of a micro-fluidic channel device [29].

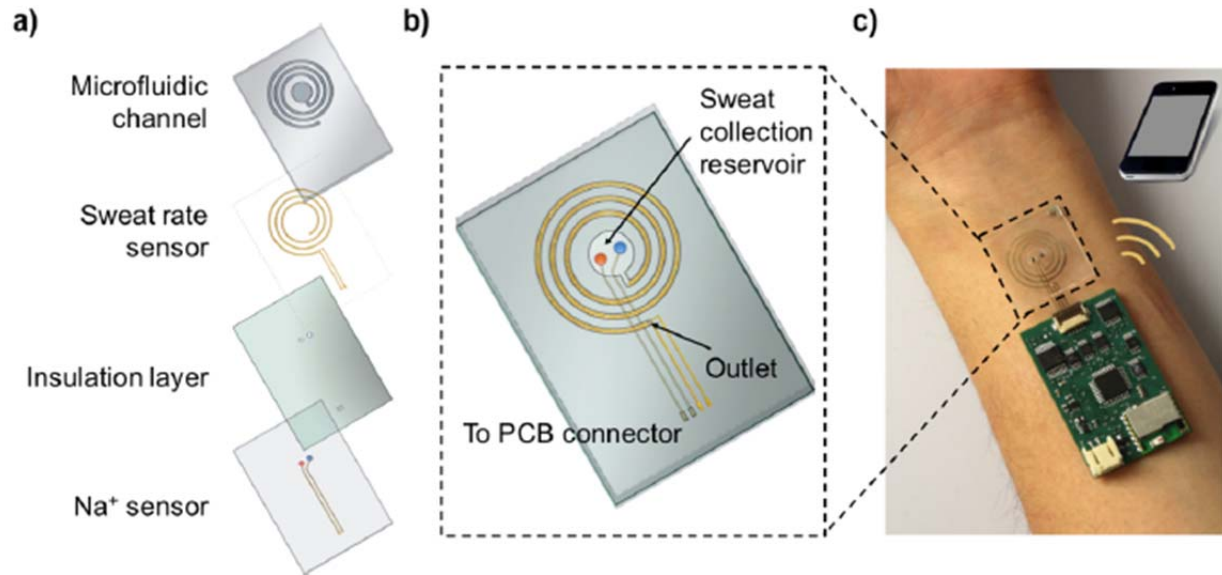


Figure 1.10. [29]. Illustrate a wearable sweat sensor. (a) a spiral-patterned microfluidic channel, Au electrodes for sweat rate sensing, an insulation layer, and Na^+ sensing electrodes. (b) Structure of the microfluidic device. The aligned Au electrodes with the microfluidic channel. The Na^+ sensor at the sweat collection reservoir. (c) The sweat sensor on human skin. The collected data from the sensor can be transferred to a cellphone through a wireless network.

In summary, it is an early stage to apply wearable sensors for ubiquitous health monitoring, but the wearable sensor technology is a rapidly growing field. The main challenge is to integrate various sensors, a battery, and wireless communication chips on a flexible substrate [30]. The separation and delamination between sensors and a substrate can be a mechanical stability issue caused by different thermal expansion and Young's modulus. Power supply and wire harness are a hurdle to be addressed. The thin film sensors measuring temperature, pressure, and strain sensor have been proven useful for health monitoring and disease diagnosis. Flexible power-generators producing electrical energy from mechanical energy can be an option to operate flexible sensors and electronics [31]. The sensors will be developed for transferring, sharing, and analyzing all data for the Internet of Things (IoT) platforms.

1.3 NON-CONTACT PRINTING METHODS

Non-contact micro and nano-printing technologies have been of great interests for various fields of electronics (**Figure 1.11**)[32] and biotechnology [33]. Major non-contact micro- and nano-printing technologies are; inkjet printing [34], pyro-electrodynamic printing [35], electro-hydrodynamic printing [36], surface acoustic wave (SAW) printing [37], dielectrophoretic (DEP) printing [38], and pen printing/deposition [39, 40]. In comparison to noncontact printing methods, contract printing methods can potentially damage underlying layers and diminish the quality of print [41]. Also, adhesion between water-based ink and the hydrophobic substrate can cause problems in relation to capillary forces [41].

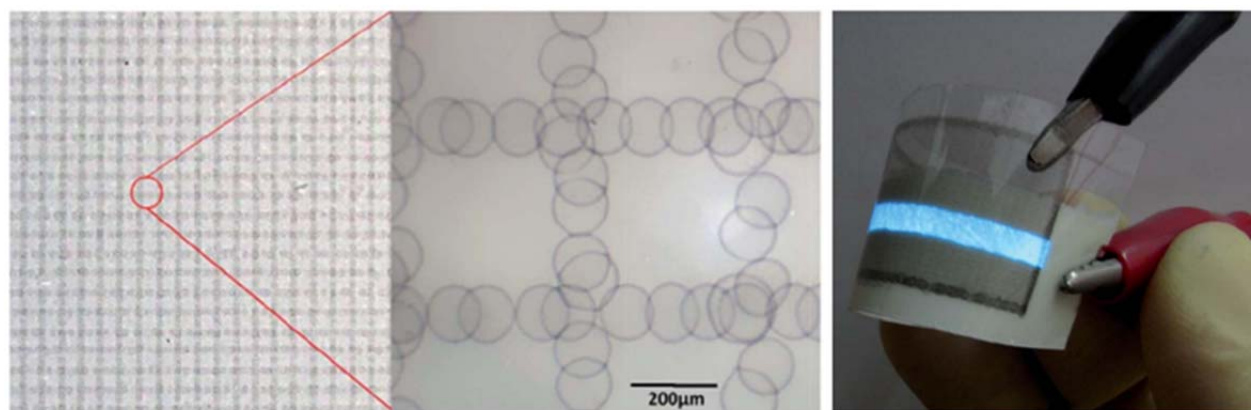


Figure 1.11. [32]. Patterned CNTs grid by inkjet printing and EL devices.

The working principles of thermal inkjet printing, piezoelectric inkjet printing, electrostatic inkjet printing, and 3D inkjet printing are similar in that the reservoir volume is

changed to eject ink. For thermal inkjet printing, flash heating is applied next to a micro-sized nozzle to induce instantaneous bubbling. The ink volume change pressurizes a portion of ink out of the chamber. The weakness in this method is that the molecules in ink should be able to withstand high temperatures. Biomolecules can be damaged at high temperature. High-Speed printing is challenging for thermal inkjet printing.

Piezoelectric printing uses the electromechanical vibration by the piezoelectric element to generate the kinetic energy of ink in a reservoir (**Figure 1.12**) [42]. The energy needs to be enough to break the surface tension in order to eject ink onto the substrate. However, piezoelectric printing is vulnerable to viscous ink because of the requirement of a higher level of energy. For high viscosity, this printing method may not be able to achieve the kinetic energy to eject an ink droplet.

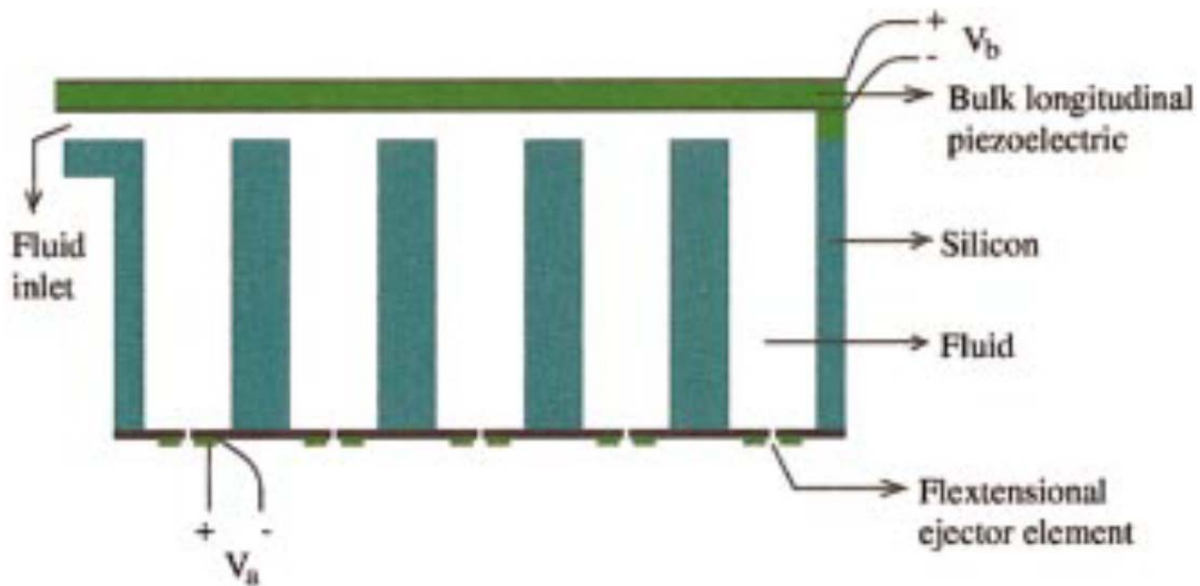


Figure 1.12. [42]. Structure of the micromachined droplet ejector.

Electrostatic inkjet printing ejects ink by the reservoir volume change [43]. The problems caused by heat or mechanical vibration can be avoided by using electrostatic force. However, working life is short [42].

3D printing dispenses polymer ink layer by layer to build a 3D object. The performance of inkjet printers is related to the viscosity of the ink and the nozzle size. Viscosity influences the printing speed as well as the material capability for printing.

All inkjet printers are influenced by ink viscosity, surface tension, particulate, and nozzle size. Particulate is a big challenge because the nozzle can be clogged by the particles in ink [44]. The resolution of inkjet printing is, therefore, unavoidably confined by particle dimensions and nozzle sizes.

Pyroelectrodynamic printing does not require electrodes or nozzles because liquid droplet is drawn from the liquid film reservoir by the pyroelectric effect. Two plates are prepared with a gap and a heat source of a heated tip in the system. A top plate is lithium niobate, and a bottom glass plate contains a liquid reservoir (**Figure 1.13**)[35]. The heat source generates thermal stimulus and occurs the electro-hydrodynamic phenomenon on the top plate by the pyroelectric effect. Local electric field exceeds a critical value and forces the ink from the top plate reservoir to be pulled on a substrate. The droplet is as small as 300 nm in radii. This printing method generates a different radius of the droplet depending on the reservoir size. The printed droplet size can be controlled, but the reservoir should be changed during the printing process. Using multiple reservoirs can address the issue of printing multiple sizes.

Pyroelectrodynamic printing is limited by the printing mechanism using heat like thermal inkjet. Heat sensitive ink material like biomaterial cannot be printed with this method. The printing speed is comparatively low due to the time to heat the substrate, though the array of the

heat source can print multiple points simultaneously.

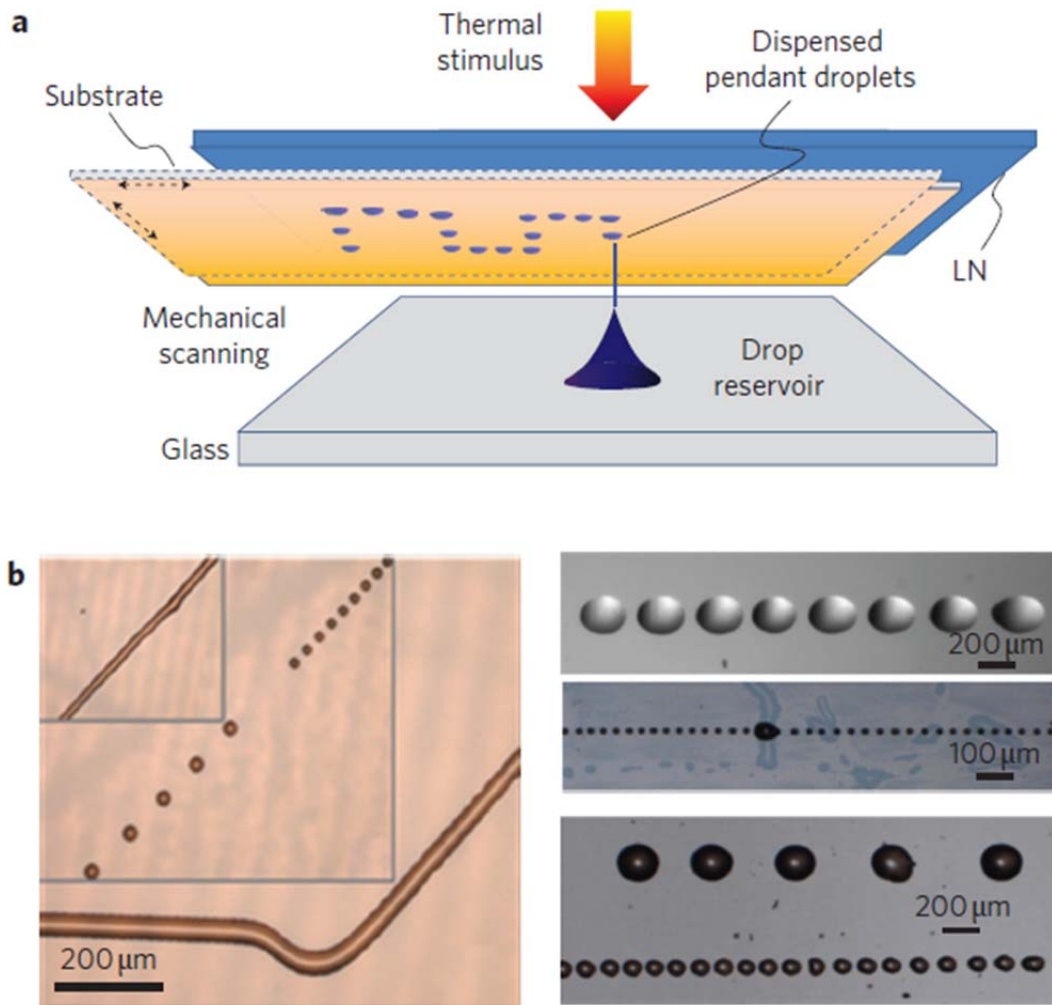


Figure 1.13. [35]. (a) Schematic image of pyroelectrodynamic printing using the LN. (c) Various patterns by pyroelectrodynamic printing, such as separate droplets, straight and curved lines.

For electrohydrodynamic (E-jet) printing, an electric field is applied to the nozzle tip and substrate to drive the ionized ink to the nozzle (**Figure 1.14**)[36]. Due to an electric field, an E-jet printing nozzle must be electrically conductive, and the substrate needs to be metallic or semiconductive.

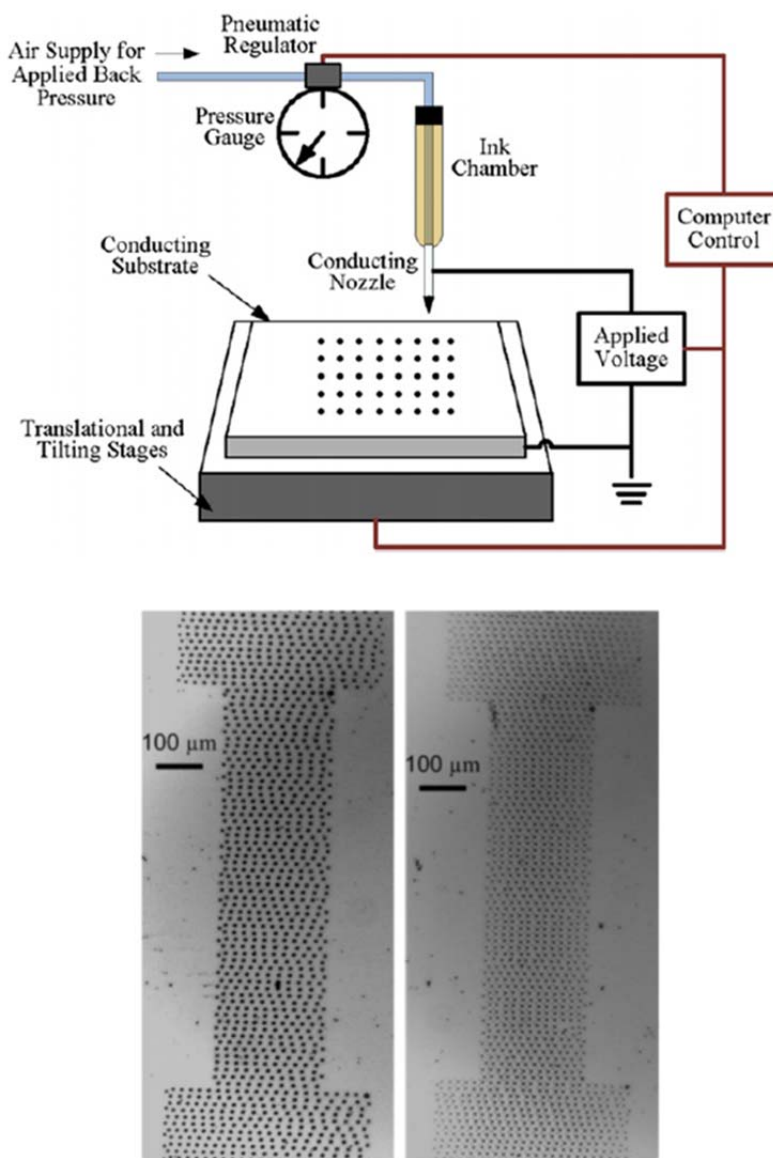


Figure 1.14. [36]. Schematic of the E-jet printing set-up (up) and printed patterns using constant voltage jetting (down left) and pulsed voltage jetting (down right).

By the electric field between the nozzle and substrate, the ions accumulate at the tip of the nozzle. Mutual Coulomb repulsion between the ions causes tangential stress on the liquid surface. The repulsion force deforms the meniscus into a conical shape called Taylor cone. The ink is printed from the tip of a Taylor cone when the electrostatic force of the ionized ink breaks

the surface tension. The droplet is smaller than the submicron nozzle size. Printing speed and droplet size can be controlled by a pulsed DC signal. Using DC pulse widths ranging from 500 μs to 2500 μs , the droplet size from 3.9 to 8.1 μm is obtained with the standard deviation of 0.4 μm and 0.3 μm .

Electrohydrodynamic printing was investigated to overcome the weaknesses of other printing methods. The potential problem of electrohydrodynamic printing lies in need of a metal/conductive printing orifice. The orifice may be affected by the ink substance. To generate an electric current, the substrate needs to be either metallic or semiconducting. A major disadvantage is to limit the printing substrate to conductive materials. The printing may not be achieved on non-conductive material such as polyethylene terephthalate (PET) film.

Surface acoustic wave (SAW) printing uses an acoustic wave field to control the fluid's movement (**Figure 1.15**)[37]. The electro-elastic wave with nanometer level amplitude propagates through the surface of an elastic substrate. This printing method is capable of nanometer resolution while either a nozzle or orifices is not required. Random patterns may not be generated by using SAW printing.

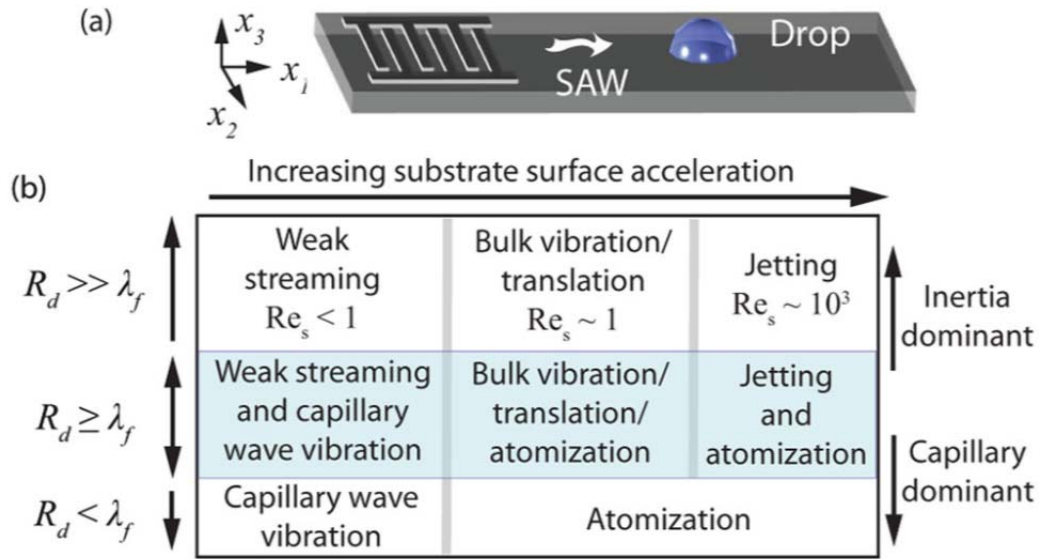


Figure 1.15. [37]. (a) Schematic view of the SAW (b) Relation between the surface acceleration magnitude and the drop size and behavior of the drop.

For dielectrophoretic (DEP) printing, DEP induces a force on the colloidal particle suspended in the liquid when DC voltage creates an electric field between the nozzle and the substrate. The DEP force results in a 175nm-size pattern of the colloidal particles on the substrate using a 150 nm nozzle (**Figure 1.16**)[38]. DEP force is subject to the dielectric constant of the liquid and the colloidal particle. The eligible printing materials are DNA [45], protein [46], the polymer [47], and metal particle. DNA, protein, and polymer materials are deposited by DEP force on the pre-patterned electrode without a nozzle

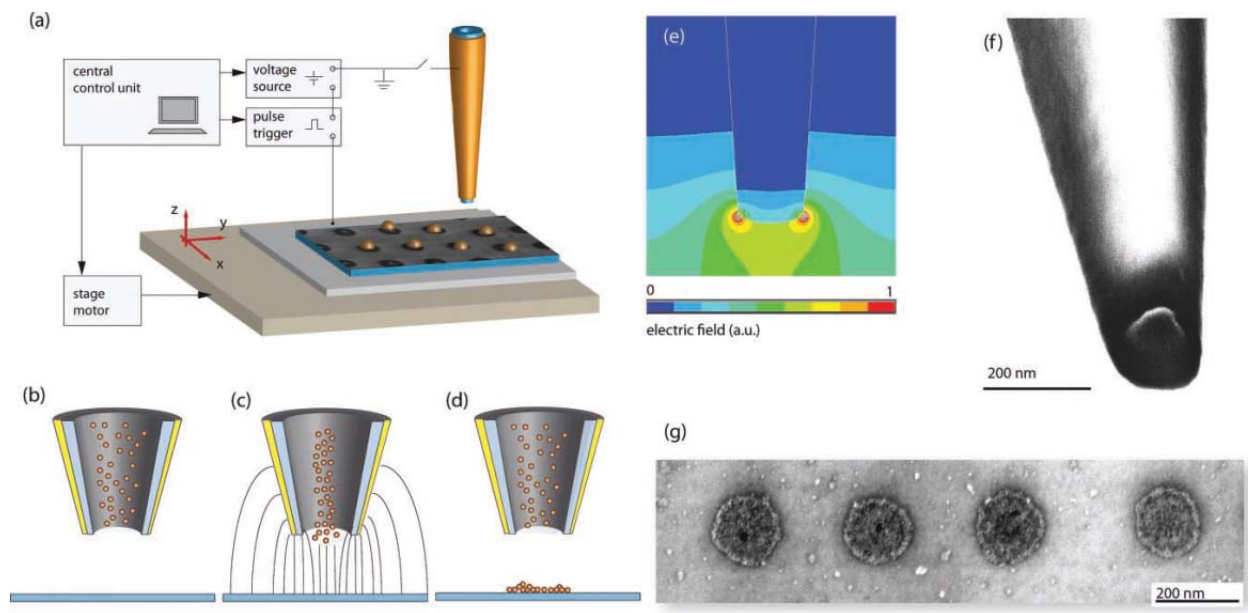


Figure 1.16. [38]. (a) Schematic of the Dielectrophoretic (DEP) printing setup. (b)–(d) The dispensing mechanism of the Dielectrophoretic (DEP) printing (e) demonstrates the electric field distribution of the nozzle. (f) Scanning electron microscopy image of glass capillary (g) Scanning electron microscopy image of corresponding Au-dots by DEP printing.

Dip-pen nanolithography utilizes capillary forces to carry molecular ink from the tip to a substrate as a nanometer size during micro-contact (**Figure 1.17**)[39]. Dip pen uses various materials such as ink, polymers [48], DNA [49], bacterial cell [50], proteins [51], and metal particles[52]. Dip Pen is influenced by viscosity, ambient conditions, adhesion properties, substrate grain size, print speed, and contact time [39]. After being dipped in ink, the pen makes a pattern on a substrate. The pattern size is related to the interaction between the ink and the substrate. The binding force between the ink and the substrate by chemisorption or self-assembly limits the diffusion length to stabilize the pattern. The printing characteristics depend on the ink properties and the substrate. Ambient environmental conditions can affect the ink evaporation causing potentially unfavorable effects like the coffee ring effect. Such printing conditions can affect the printing resolution.

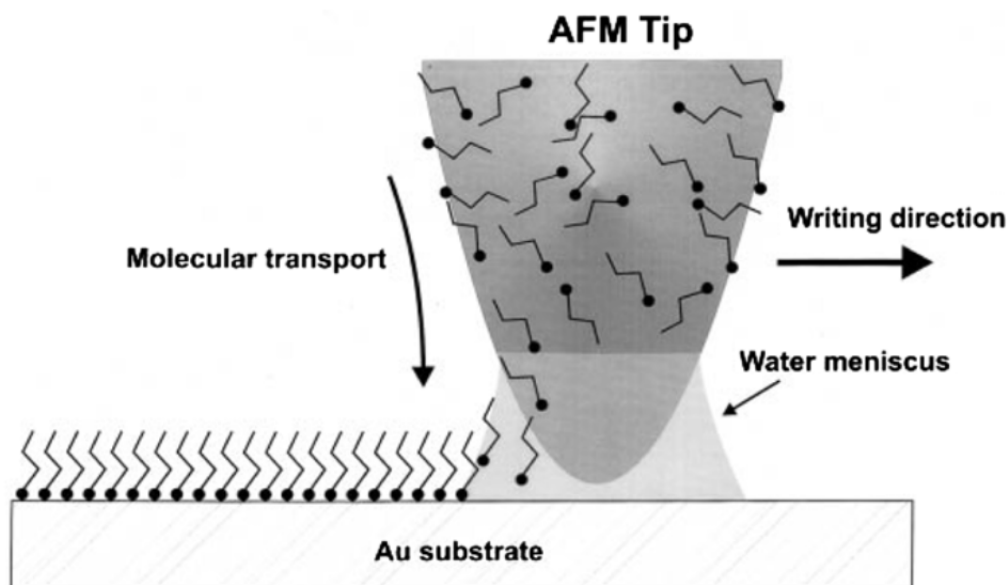


Figure 1.17. [39]. Illustration of dip-pen nanolithography (DPN) writing.

A nano fountain pen method uses a tapered pen tip that can be fabricated with a heat-drawn hollow glass or quartz. Ink is delivered through a channel of a few hundred nanometers onto a substrate by capillary [40, 53, 54]. The liquid droplet stays on the tip by surface tension until the tip is in contact with the substrate. The ambient environmental condition affects the drying condition of the water-based ink. The condition causes the coffee ring effect to the printed pattern like dip pen lithography [55]. Polymer [56], metal [57], carbon nanotube [58], and protein [40], have been patterned by a nano fountain pen.

Printing method	Precision	Nozzle	Nozzle structure	Contact	Printable materials
Thermal inkjet	Micron	Yes	Complex	Non-contact	polymer, metal
Piezoelectric inkjet					polymer, metal
3-D inkjet					polymer, ceramic, glass
Electrostatic inkjet	Submicron		Simple		polymer, metal
Electrohydrodynamic					polymer, DNA, protein, metal
Dielectrophoretic					polymer, DNA, protein, metal
Pyroelectrodynamic		nanotubes, nanowires			
SAW		polymer, metal			
Dip-pen nanolithography		No		Contact	polymer, DNA, protein, metal
Nano fountain pen	polymer, DNA, protein, metal				

Table 1. [42]. Characteristics of printing methods.

All the printing methods are summarized in (**Table 1**) [42]. Inkjet printers are widely used in comparison to other printing methods, though the inkjet resolution has micron precision. The complicated structure of the nozzle is fabricated by the semiconductor fabrication process. In spite of scalable production capability, the cost of the semiconductor process is high. Ink materials affected by heat or mechanical vibration may not be allowed for inkjet printing. Dip-pen nanolithography and nano fountain pen are capable of using various ink materials, but these printing methods cause substrate damage because of physical contact in printing [59].

1.4 CHALLENGES

To print multiple layers of different nanomaterials on a 2-D substrate, various approaches have been investigated. Among them, non-contact printing is preferred to avoid potential damage to the substrate or the previously-printed layers. Inkjet-style printing [60-62] is the most common basis of various commercially available techniques. Thermal and piezoelectric inkjet printing methods use thermal expansion and electromechanical vibration to eject droplets, overcoming surface tension in the formation of droplets [60, 63]. Due to the physics of droplet formation, inkjet printing is limited in the range of viscosities and values of surface tension of the working fluid [64, 65]. When the nano-ink of interest has high surface tension and low viscosity, cumbersome change of ink properties is performed to generate a stable droplet [32, 66, 67].

Additionally, fragile biomolecules are not compatible with the high pressures, heat, and shear stresses associated with printing through inkjet methods [64, 68, 69]. Electrohydrodynamic printing utilizes an electric field to accurately control flow through a nozzle [70-72] but is limited to conductive or semi-conductive substrates. Pyroelectrodynamic printing uses the pyroelectric effect to cause the localized electric charge to pull drops to the substrate [73, 74]. Dielectrophoretic printing [38] and surface acoustic wave printing [37] use electric- and acoustic fields, respectively. However, printing capabilities on a 3-D surface have not been reliable. Stencil printing [75] can spray nanomaterials through a mask pattern but is limited in random patterning.

As an alternative to current nanoparticle printing methods, fountain pens [76-78], ball pens [79] and pencils [80, 81] have been demonstrated in nanomaterial printing. However, these contact printing methods could cause damage to the substrate [78], which impedes the printing of multiple layers or large scales. For nanoscale patterning, dip-pen lithography [39, 82] has been

developed for molecular printing but has limitations in terms of scalability and nano ink delivery. When using capillary pens, the nano-ink can evaporate and dry at high rates inside the capillary tube or needle, which restricts their use to very short times. This represents a major hurdle for scalable printing of molecules.

1.5 OBJECTIVES

To address the main challenge of SWCNT printing on a film, we study capillary pen printing through a nano ink bridge. The presented approach would avoid the drawbacks of surface damage due to the contact of the pen's nib on the substrate and enable the patterning of multiple layers. In particular, we propose to use a stylographic pen that consists of a capillary nozzle and a rod-shaped ink stopper.

The proposed study consists of two steps. The first is to investigate the effect of varying control parameters on the uniformity of printed features, such as width, edge roughness, and sheet resistance of the printed line on a 2-D surface. To enhance the uniformity of the printed line, the substrate can be heated to control the contact angle and evaporation of the solution. Through the dot test, the spread of the solution and the contact angle is measured under a different temperature condition of the substrate. And then, the line is drawn by the Rotring pen. The contact angle is the key parameter for the printed line property.

Using printed SWCNTs, chemical sensors will be developed. The existing printing method is challenging to fabricate continuous line and multiple layers for doping with chemicals because of the nature of discrete drop printing. To make a continuous line, the printing needs to

be repeated with alignment. Using the proposed printing methods, gas and ion sensors will be developed.

Chapter 2. NANOINK BRIDGE-INDUCED CAPILLARY PEN PRINTING

2.1 INTRODUCTION

Since the discovery of carbon nanotubes (CNTs), various patterning methods have been investigated to develop chemical and biological sensors. Early methods relied on direct growth on a substrate, assembly using an electric field, and self-assembly [83-85]. In the fabrication of wearable sensors, a non-contact printing is preferred to avoid potential damage to the substrate or existing layers. Mostly inkjet printings [60-62] utilize the principle of the thermal expansion or electromechanical vibration [60, 63]. Formation of the droplet relies on the viscosity and surface tension [64, 65], which often requires significant modifications of ink properties [32, 66, 67]. To achieve electrical conductance of the printed CNT patterns, the discrete nature of droplet deposition requires the ejector shift to connect the drops. In the printing of biological materials, it may not be usable because the high pressures, heat, and shear stresses in inkjet reservoir could damage the fragile biomolecules [64, 68, 69]. Electrohydrodynamic printing makes use of an electric field to eject ink through a nozzle [70-72], so conductive and semi-conductive substrate have to be used. Stencil printing [75] deposits nanomaterial by spraying through an open stencil mask, which is limited to the designed masking pattern.

Fountain pens [76-78], ball pens [79], and pencils [80, 81] have been researched to pattern nanomaterials. For fountain pens, the capillary force attracts ink into the tube and provides the

pressure gradient to hold ink column inside. When the capillary tube is in contact with a porous paper, the capillary pressure in pen decreases due to the contact between the nib and the porous substrate, resulting in ink flow [86]. However, printing on an impermeable film is more challenging as the contact angle is much greater than 0° . Moreover, any contact printing may damage the substrate [78] hindering multiple layer deposition required for complex sensor structures.

2.2 OBJECTIVES

In this chapter, a noncontact capillary pen printing method is characterized. The technique is demonstrated by patterning single-walled carbon nanotubes (SWCNTs) via a nano ink liquid bridge. The non-contact printing method does not physically damage the substrate or previously deposited layers. A single pass printing is sufficient to obtain a measurable electrical resistance. The resistance and the optical properties are characterized for several print geometries: a dot, a line, and a film.

2.3 NANOINK BRIDGE-INDUCED PRINTING

The noncontact capillary method deposits nano ink through a liquid bridge forming between a capillary pen and substrate (**Figure 2.1a**). A stylographic pen consists of a capillary nozzle and a rod-shaped ink stopper that assures nano ink seal when the pen is not used. During

printing, two geometric parameters require control in order to maintain the capillary bridge integrity: the pen tip height (H) from the substrate and the advancing bridge contact angle (θ_{B_a}) as illustrated in (**Figure 2.1a**). When the liquid bridge is established, the ink flow rate depends on the pressure difference between the capillary pen reservoir and the substrate surface. In a static condition, θ_{B_a} is dependent on the substrate surface properties, its temperature, and ink properties. When the pen moves to the right, θ_{B_a} increases, and the receding angle (θ_{B_r}) decreases. As θ_{B_a} increases, the hydrostatic pressure on surface increases to reduce the ink flow. For low θ_{B_a} , the pressure difference is maximized, resulting in the high ink feed rate.

(**Figure 2.1b**) shows the printing setup consisting of an x-y-z plotter and control module. The printing direction is controlled by two step motors in x and y directions. Manual micropositioning stage sets the Z-coordinate (pen tip height; H) necessary to form a nano ink bridge. A camera with a microscopic objective lens monitors the condition of the liquid bridge for feedback control. The substrate temperature is controlled via closed-loop by a thermocouple as a sensor for a heating stage.

(**Figure 2.1c**) illustrates the printing procedure. First, the nib is pressed in the axial direction, and nano ink is released due to capillary action. Second, upon the release of the nano-ink, the pen is withdrawn from the substrate to $H = 100 \mu\text{m}$; nano ink bridge forms between the pen tip and the substrate. When the pen moves to the right, θ_{B_a} increases and θ_{B_r} decreased. Finally, the pen is retracted to stop the print. (**Figure 2.1d**) shows a typical example of the printed pattern using the nano ink-bridge printing on a polyethylene terephthalate (PET) film. A PET film is an appropriate substrate for chemical sensors because it is chemically resistant, transparent, and mechanically robust.

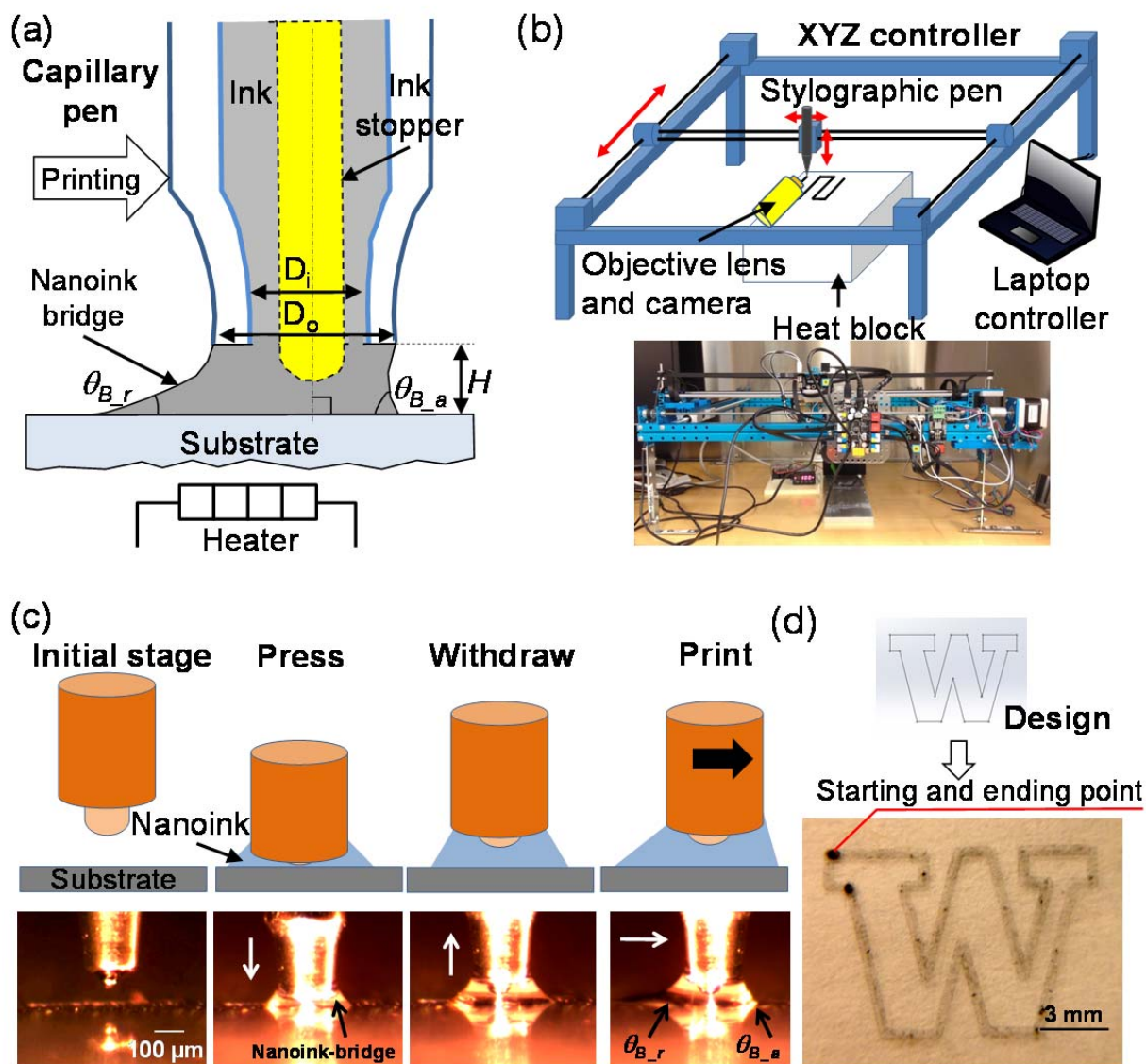


Figure 2.1. Nanoink bridge-induced capillary printing (a) Printing concept (b) Schematic of an XYZ plotter installed with a heating stage and a camera system. The top image shows a printing system, and the bottom is a photograph of the setup. (c) Nanoink-bridge induced printing using water ink on a PET film. The ink is released with pressing the stopper. Upon withdrawal by 100 μ m, an ink bridge forms. The advancing contact angle increases as the pen moves from left to right. (d) W-pattern printed by SWCNT-ink at 80°C at 1.2 mm/sec. The top image shows a design.

2.4 EXPERIMENTAL METHODS

2.4.1 *Characterization of the printing method*

To study the printing characteristics, a dot, a line and a film of SWCNTs were printed at various substrate temperatures and printing speeds. Nanoink was prepared by suspending SWCNTs (5 mg/mL) in 1 % sodium dodecyl sulfate (SDS) by sonication. In the suspension, the supernatant was used as nano-ink. The advancing contact angle (θ_{B_a}) on a PET film was measured for various substrate temperatures and printing speeds. The morphology of the printed patterns and the electrical properties were characterized. The nominal diameters of the capillary pens were 100, 300, and 700 μm . The outer diameters of the pen nib (D_o in **Figure 2.1a**) were 225, 375, and 790 μm , respectively (**Figure 2.2**). The outer diameters determined the minimum line width in printing as the meniscus attached to the outer dimension of the nib. In the paper, the nominal diameters are used hereafter.

Dot printing A single dot was printed at various substrate temperatures 20 °C, 40 °C, 60 °C, 80 °C, and 100 °C using a 300 μm -diameter pen. The pen reservoir was filled with SWCNT-ink and then installed on a printer. The pen was pressed and then withdrawn to $H = 100 \mu\text{m}$ to form a nano ink bridge. To study the evaporation characteristics, the holding time was controlled at 1, 5, and 10 seconds at each temperature. The contact angles (θ_B) were measured for each case by using the camera images in the printer. The height profile of the dots was scanned by a profilometer (Alpha-step D-300 stylus profiler, KLA-Tencor Corporation).

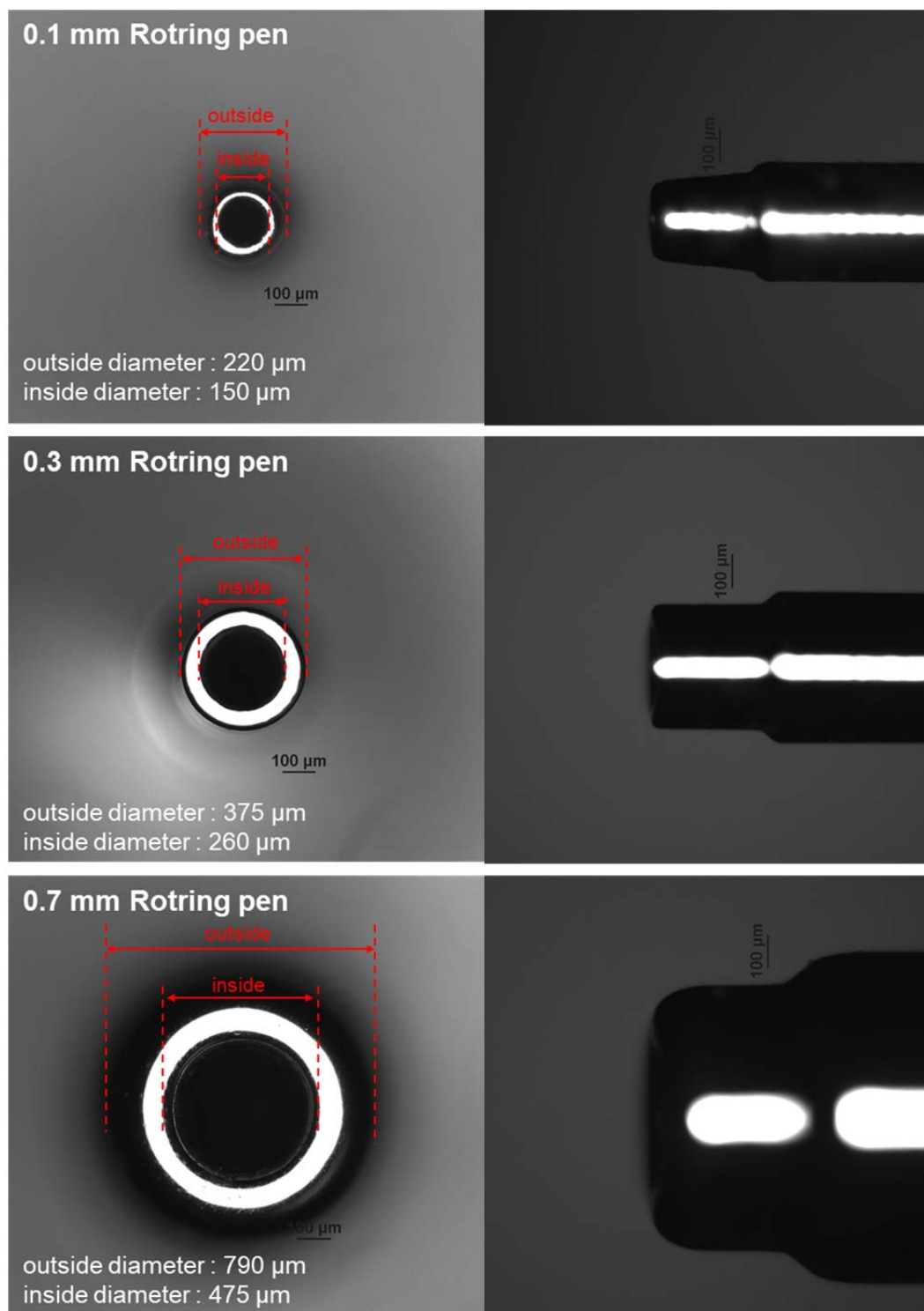


Figure 2.2. Diameters of the capillary pens. The nominal diameters of the capillary pens are 100, 300, and 700 μm . The outer diameters of the pen nib are 225, 375, and 790 μm , respectively.

Line printing To print a line, numerical control (G-code) was used to control x and y directional step motors. A 100 μm capillary pen was used. After the nano-ink bridge formed at a 100 μm gap, a straight line was deposited on a PET film. Printing speed was set in the range from 0.2 to 10 mm/s, and the temperature was controlled from 20 $^{\circ}\text{C}$ to 100 $^{\circ}\text{C}$. The line width and θ_{B_a} were measured for each speed and temperature. After printing, a silver paste was applied to both ends of the line to form electrodes. A picoammeter (6487 Picoammeter/Voltage Source, Keithley Instruments) was used to measure current-voltage (I - V) characteristics of the printed line. The printed line was imaged by an optical microscope (Olympus BX-41, Olympus, Gaithersburg, MD, USA) and scanning electron microscopy (SEM) in order to characterize the morphology of SWCNT lines.

Film printing Film printing was achieved by drawing continuous lines in linear hatch pattern covering the area of 15x15 mm². Printing speed was set for 2.5 mm/sec, and the substrate temperature was varied from 20 to 100 $^{\circ}\text{C}$. The nominal diameter of a capillary pen was 700 μm . To achieve complete coverage, the pen was shifted by 600 μm for each pass (total of 25 parallel passes). Optical transparency measurements were performed by transmission optical microscopy (Olympus BX-41, Olympus, Gaithersburg, MD, USA). The transparency was computed as a ratio of the transmitted white light intensity through the printed area over the non-printed area. The sheet resistance was measured using a custom 4-point probe measurement system (**Figure 2.3**).

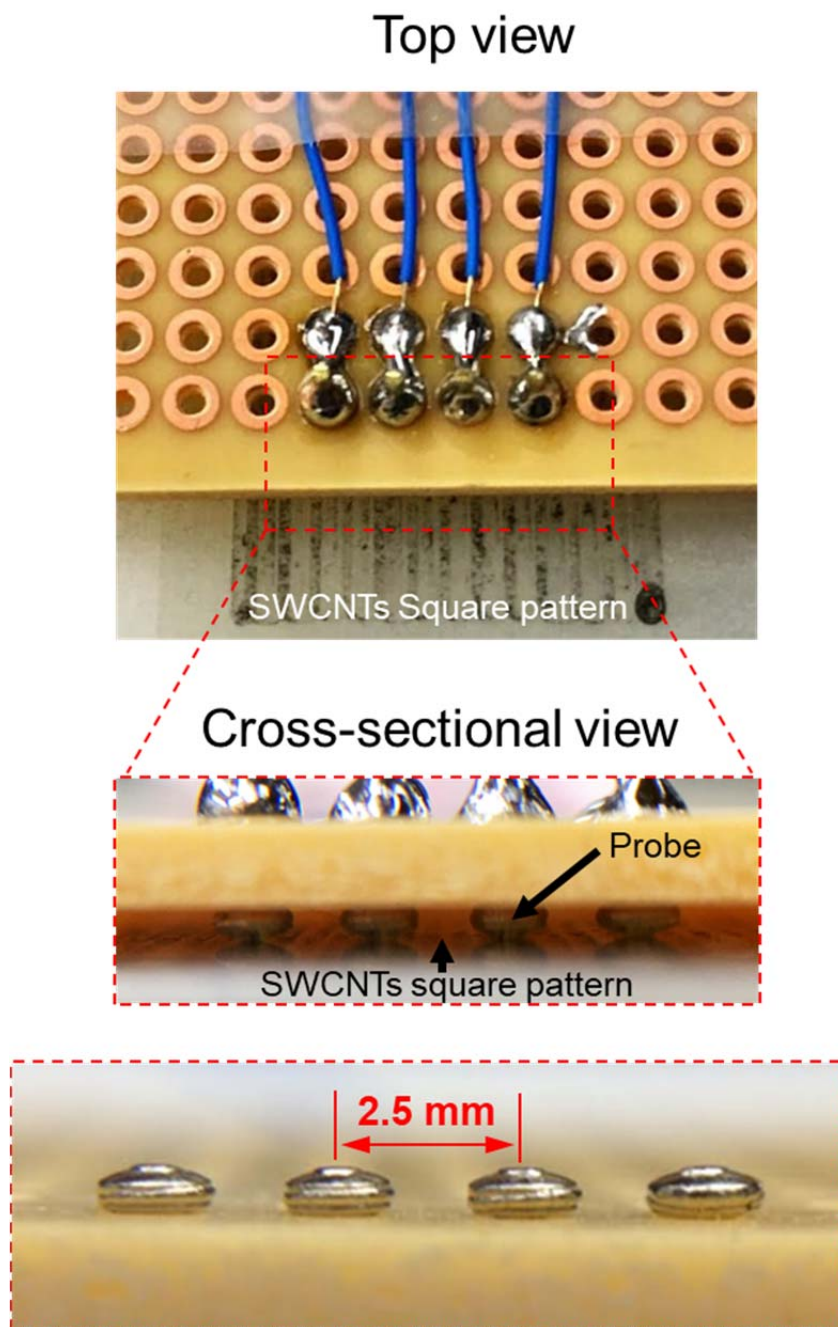


Figure 2.3. 4-point probe measurement setup. The sheet resistance is measured using a custom 4-point probe measurement system. The distance between electrodes is 2.5 mm.

2.4.2 *Doping effect*

The electrical characteristics of SWCNT lines were studied for doping polyethyleneimine (1 % PEI, Fluka). The printing sequence was varied for PEI and SWCNTs. In one case, SWCNT lines were printed first, followed by PEI deposition (SWCNT/PEI). In the other case, the order was reversed. The SWCNT lines were printed on top of PEI (PEI/SWCNT). The printing conditions were 80 °C with printing speed of 0.83 mm/sec where a stable line patterning could be obtained. Silver electrodes were patterned on the SWCNT lines. For both cases of SWCNT/PEI and PEI/SWCNT depositions, PEI solution (1 μ L) was dispensed by 1, 2, and 3 times in order to analyze a doping effect and electrical stability. After each deposition, PEI was cured in a convection oven at 100 °C for 1 hour. *I-V* characteristics were measured for all cases.

2.5 RESULTS

2.5.1 *Characterization of the printing method*

Dot printing Dot printed patterns were analyzed to determine nano ink behavior at various temperatures and holding times. A circular dot printed patterns formed when a capillary pen was pressed and retracted to 100 μ m on a PET film for 1, 5, and 10 seconds (**Figure 2.4a**). As the temperature increased, the ratio of the dot diameter to the outer nib diameter decreased and approached unity (**Figure 2.4b**). The dot size reduction was attributed to the increase of the

static contact angle (θ_{B_a}) and the pinning at the meniscus due to ink evaporation on the hot surface. As the substrate temperature increased, the surface tension and the viscosity decreased. However, the evaporation rate increased θ_{B_a} (**Figure 2.4c**). As θ_{B_a} approached 90° , the meniscus edge was pinned yielding the ratio of unity. As the substrate temperature increased, the drop spreading on the substrate was reduced, suggesting the effects of the increased evaporation rate at the drop edge.

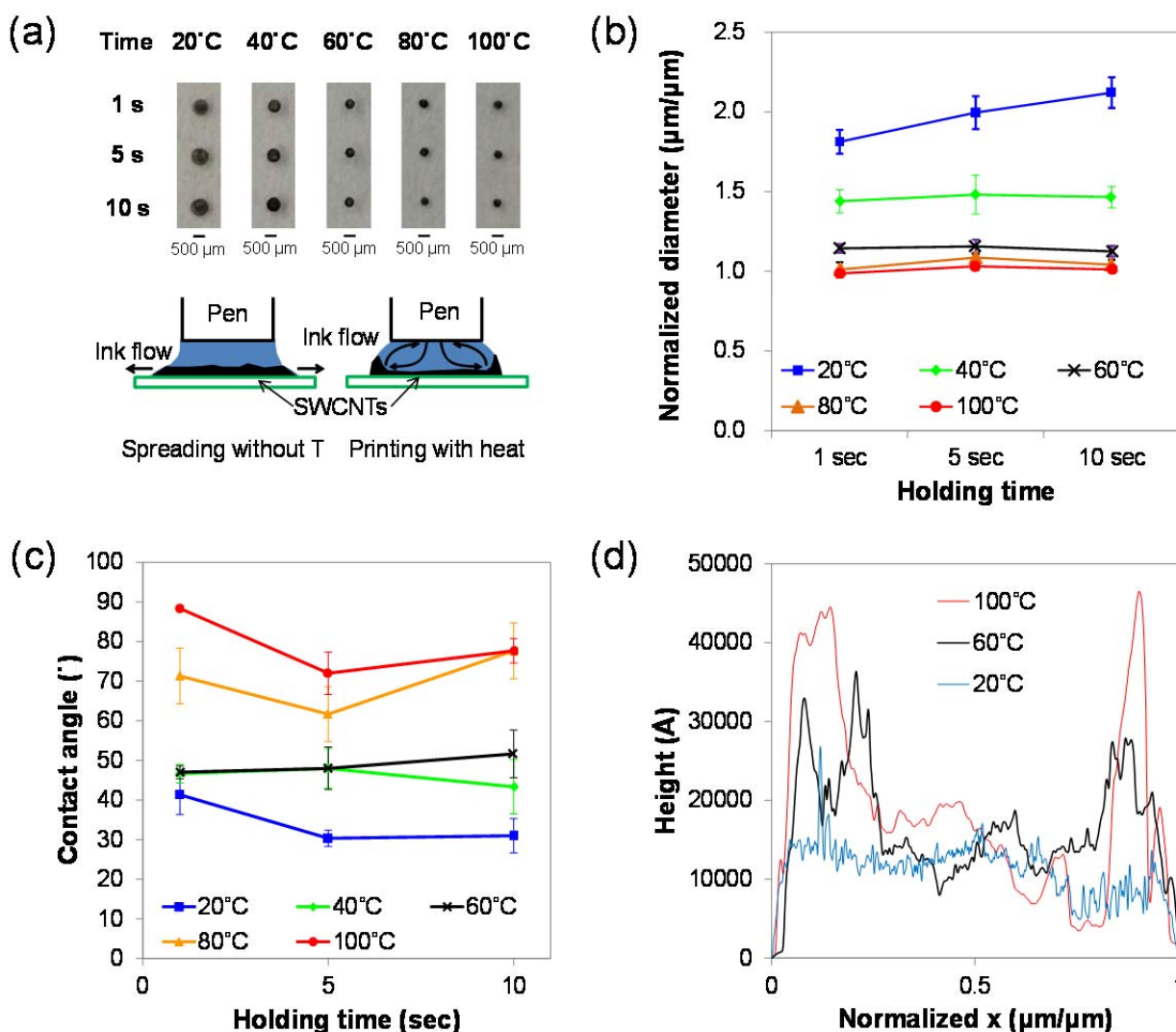


Figure 2.4. Dot printing (a) Dots printed at surface temperature 20~100°C with holding time of 1, 5, and 10 s. Scale bar: 500μm (b) Dot diameters normalized by an outer nib diameter (c) Contact angles according to holding time (d) Dot profile at 20, 60, and 100°C (1s holding time).

Profilometer measurements of the dot depositions show that a coffee-ring effect is present for a higher substrate temperature (**Figure 2.4d**). The greatest deposition height occurred for prints with the surface temperature of 100 °C. During the deposition, SWCNTs were continuously delivered to the edge of the ink bridge where the liquid evaporation rate was the greatest, resulting in an increased local concentration of the SWCNTs in the solution, thus their thickest deposition at the dot edges. At room temperature, relatively uniform distribution of SWCNT ink was observed as the ink flow rate, and its deposition rate was balanced by the evaporation rate.

Line printing Microscopic observation of the printed line patterns shows that three distinct printing regions exist: (Region 1) print line width decreases with the increase of print speed in the low-temperature prints (20 and 40 °C); (Region 2) line width is constant at medium temperature (60 and 80 °C); (Region 3) line width increases with print speed – at high temperature (100 °C) and lower speeds. The trend is due to changing fluid properties and θ_{B_a} .

(Region 1) was observed at the lower temperature conditions (20 and 40 °C) and medium temperature (60 °C) at higher print speeds; the line width reduced with the increase in print speed and the increase of the substrate temperature (**Figure 2.5a** and **2.5b**). At room temperature, the normalized line width, which is the ratio of the printed line width to the actual outside diameter of a Rotring pen, was reduced from 2.5 to 1 as the print speed increased from 0.2 to 2.5 mm/s. The nominal and actual diameters are given in the (**Figure 2.2**). At the low print speed, low contact angle, and low shear stress, the line width was not uniform, indicating the unstable behavior of the capillary bridge. As the print speed increased both shear stress and contact angle, the bridge was stabilized, resulting in the stable printing conditions exhibiting

constant line width and lower flow rate, which was also consistent with the increased sheet resistance. The reduction of the line width suggests that (i) the increased θ_{B_a} (**Figure 2.5c**) causes the increase in the contact angle on the sides of the droplet to parallel to the nib motion due to the surface tension, (ii) capillary bridge elongated along the printing direction with the shear stress. For the lower temperature condition, the prints failed at the speeds > 2.5 mm/s, as θ_{B_a} approaches 90° .

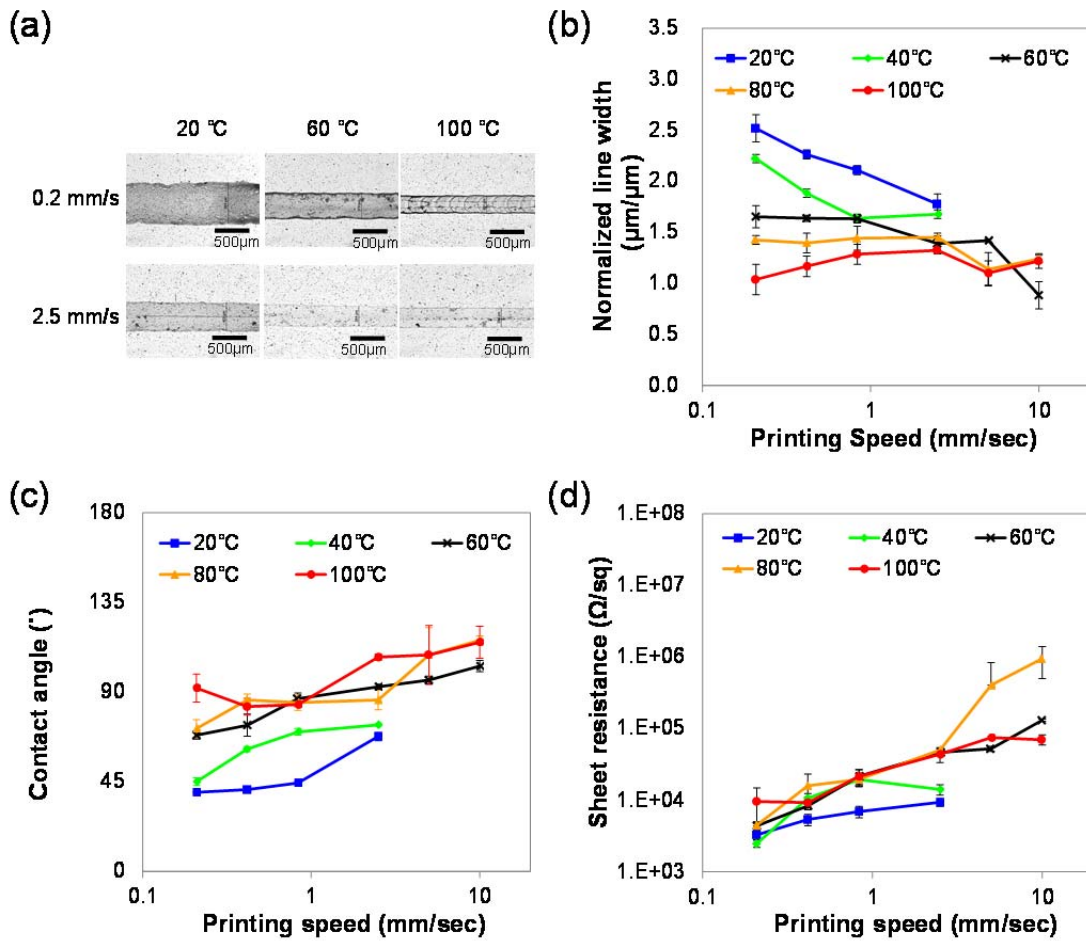


Figure 2.5. Line printing (a) Printed lines at 0.2 and 2.5 mm/s under the substrate temperature of 20~100 °C. (b) Normalized line widths at a temperature of 20~100 °C with a printing speed of 0.2~10mm. (c) Advancing contact angle (θ_{B_a}) at various printing speed. (d) Sheet resistance according to print temperature and speed.

(Region 2) was characterized by relatively constant line width independent of the print speed at 60 and 80 °C. At the higher temperatures, the ink viscosity reduced, resulting in the lower local shear stress allowing the ink to flow more uniformly at the larger contact angle. Experimentally the print speed increasing from 2.5 mm/s to 10 mm/s did not compromise the bridge stability though the advancing angle exceeded 90 ° for the faster prints. The high θ_{B_a} resulted in the high and stable contact angles on both sides of the bridge.

(Region 3) was characterized by the increase in the line width as print speed increased at $T=100$ °C and print speed below 2.5 mm/s. It is speculated that the effect was caused by the high rate of ink evaporation at the low print speeds as the liquid in the bridge approached its boiling point. As the speed increased, the time allowed for evaporation reduced. The ink was delivered to the substrate more effectively resulting in a wider print line. At the higher speeds (>2.5 mm/s), the normalized line width reduced, which was consistent with the increase of θ_{B_a} as shown in **(Figure 2.5c)**.

Related to the optimization of the print conditions: at the temperatures greater than 60 °C, SWCNTs could be printed as $\theta_{B_a} > 90^\circ$. At the temperature of 80 and 100 °C, a stick-slip effect was observed at the advancing meniscus of a nano ink bridge. The beach mark pattern in **(Figure 2.5a)** and an SEM study were consistent with the stick-slip effect **(Figure 2.6)**. Similar to the dot printing, the printed lines showed a coffee-ring effect at the temperature > 60 °C while a flat profile was observed at the temperature < 60 °C. The thickness of the printed line was well below 1 μm , which could not be measured by a profilometer or an atomic force microscope (AFM) because of the relatively rough PET surface. The formation of the coffee ring could be observed by the higher contrast in an optical microscope and SEM **(Figure 2.6)**.

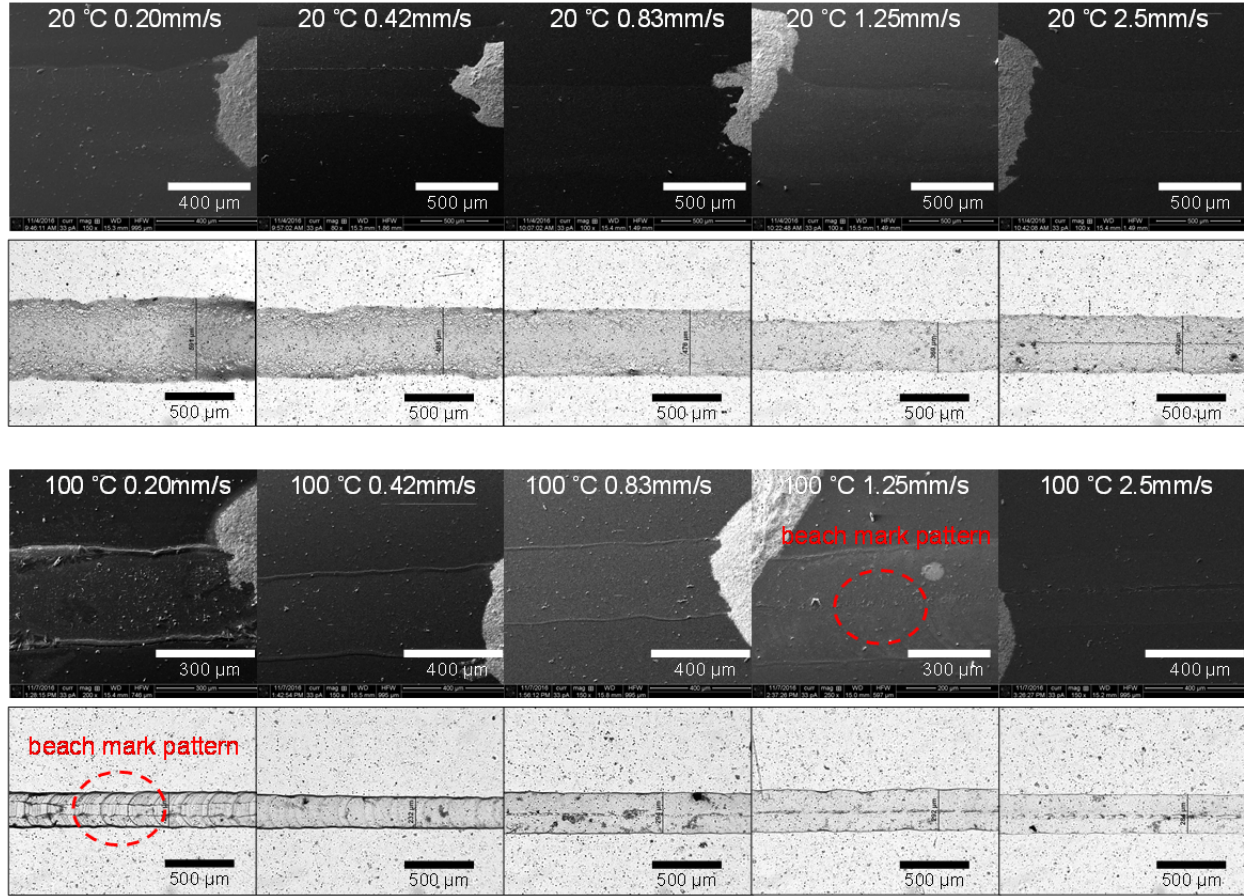


Figure 2.6. Printed SWCNT lines at 20 and 100 °C. Top rows are SEM images, and bottom rows are optical microscope images for 20 and 100 °C, respectively. The beach-mark pattern is observed at 100 °C due to a stick and slip effect. The two lines at the edge of an SWCNT line at 100 °C form by a coffee ring effect.

For electrical characterization of the prints, a silver electrode was patterned at the ends of a printed line. In comparison to 4-point probe measurement, the contact resistance using the 2-silver electrodes was only $9.6 \pm 0.6 \%$, which was consistent throughout multiple measurements ($N = 6$). The I - V characteristics showed a linear trend due to the metallic SWCNTs in the printed lines. The sheet resistance of the lines became larger as both temperature and speed became higher (**Figure 2.5d**). With the increase of $\theta_{B,a}$, the smaller pressure difference reduced the flow rate of SWCNT ink, which increased the sheet resistance.

Overall, the increase in the print speed yielded the greater sheet resistance as a result of the higher contact angle and reduced flow rate. At the higher speed, the contact angle increased the pressure on the substrate, which resulted in the smaller nano ink flow rate thus the reduction of SWCNTs deposition and greater transparency of the print (**Figure 2.7**). Unlike in the inkjet printing, both desired transparency and electrical resistance could be obtained with a single print, which shows the advantage of the nano-ink bridge induced capillary printing.

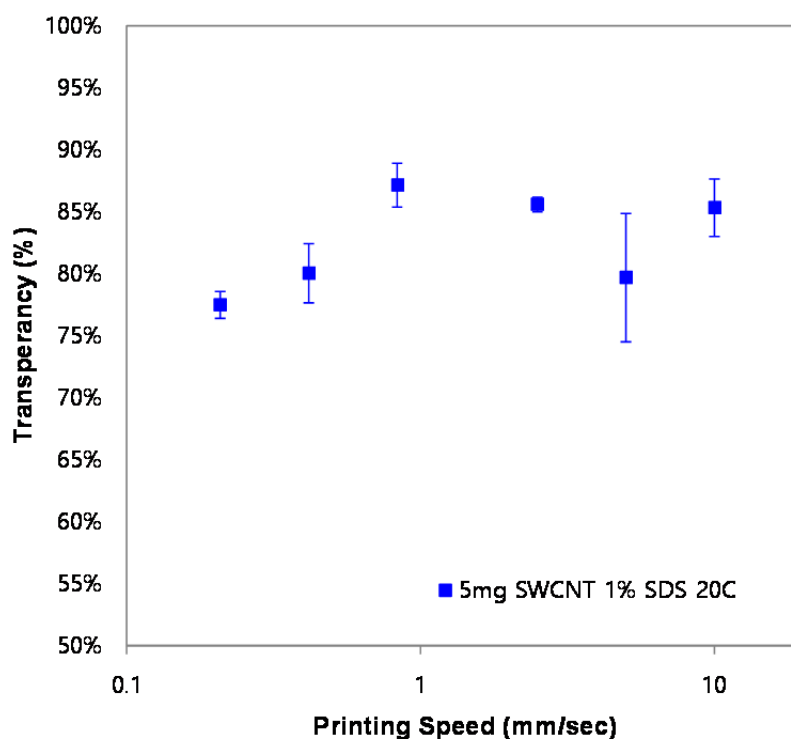


Figure 2.7. Transparency for an SWCNT line according to various printing speed at 20°C.

Film printing For film printing, the area of 15x15 mm² was printed in 3 minutes with the nib speed of 2.5 mm/sec. When the printing lines were overlapped at T = 20°C, the nano-ink was smudged across the printed lines. The optical transparency of the film increased from 67 % to 89 % as the temperature increased (**Figure 2.8a**). For print temperatures > 60 °C, SWCNT clusters were observed; during printing, the SWCNTs were aggregated at the stopper, leaving clusters on the substrate. The sheet resistance increased from 2.5 to 62.4 kΩ/sq as the temperature increased (**Figure 2.8b**), due to the larger bridge contact angles and thus, the reduced ink flow rate. The sheet resistance was the highest at 80 °C and reduced at 100 °C, consistent with line printing observations.

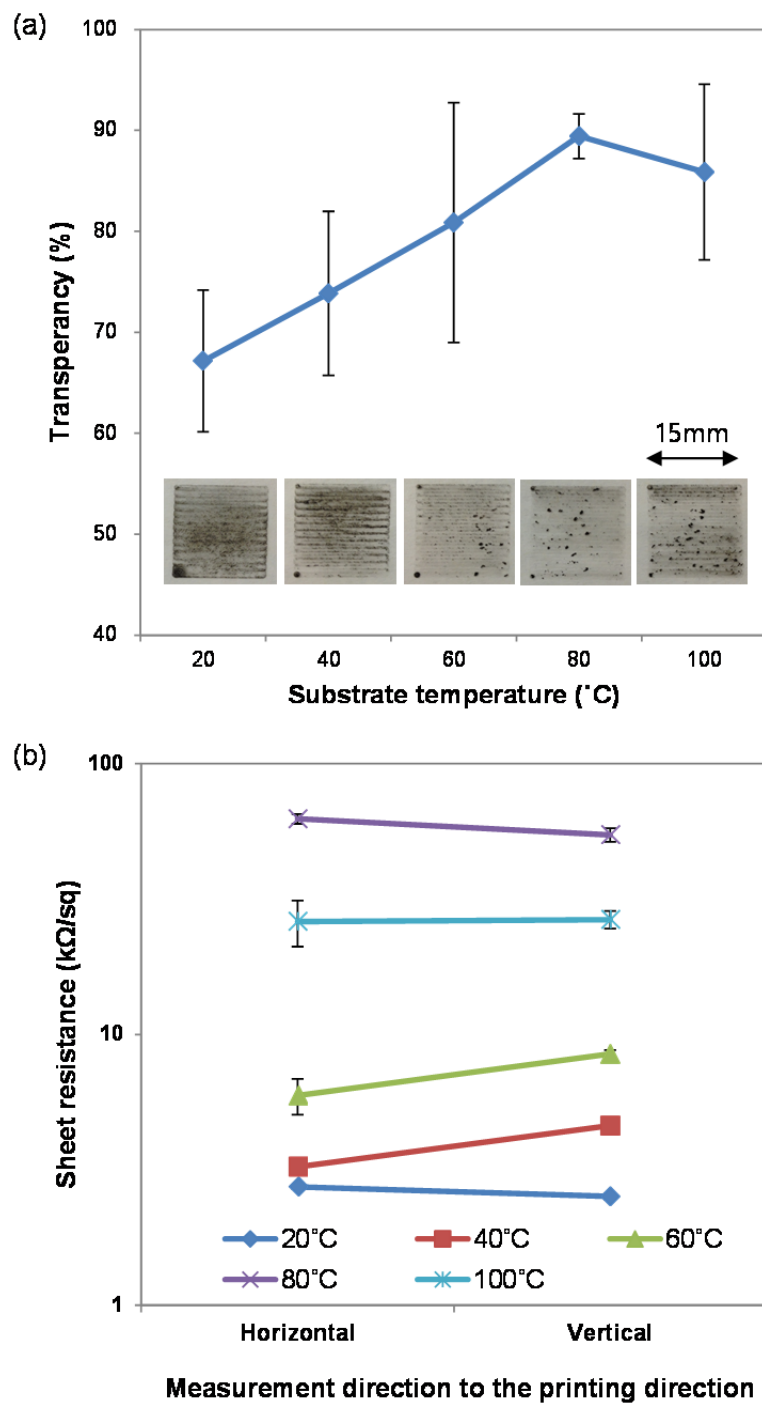


Figure 2.8. (a) Transpacency of printed films at temperatures of 20~100°C. Printing speed: 1 mm/s (b) Sheet resistance in the printing and its vertical directions.

2.5.2 Doping effect

The doping effect of SWCNTs was studied by using PEI. PEI was deposited before or after SWCNT printing. For SWCNT/PEI lines, the current decreased as the number of PEI deposition layers increased (**Figure 2.9a**) based on *I-V* characterization (inset Figure of **Figure 2.9a**). For the repeated tests ($N = 3$), the error bars for the electric current showed ± 9.5 , ± 6.8 , ± 5.3 , ± 1.6 % for 0, 1, 2, and 3 depositions. With more depositions, the error bars were reduced because semiconducting SWCNTs with various chirality reached current saturation by the doping. Considering the initial p-type SWCNTs, the current decreased due to the PEI' amine group doping. For measurement over 1000 s, the resistance change was only ± 0.09 %, showing layer stability (**Figure 2.9b**).

For PEI/SWCNT lines, the doping effect was not as reproducible as the SWCNT/PEI lines (**Figure 2.9c**): the SWCNTs might not be fully covered with PEI. The resistance could increase or decrease for forward and backward printing directions (**Figure 2.9d**). The large change in the resistance appeared to be related to the physisorption and reaction with air. When the SWCNT device was placed in a vacuum chamber (125 mmHg), the resistance was constant because of the low oxygen environment (**Figure 2.9d**). (**Figure 2.9c** and **2.9d**) suggest that the *I-V* nonlinearity resulted from the continuous change of PEI/SWCNT resistance due to the interaction with air.

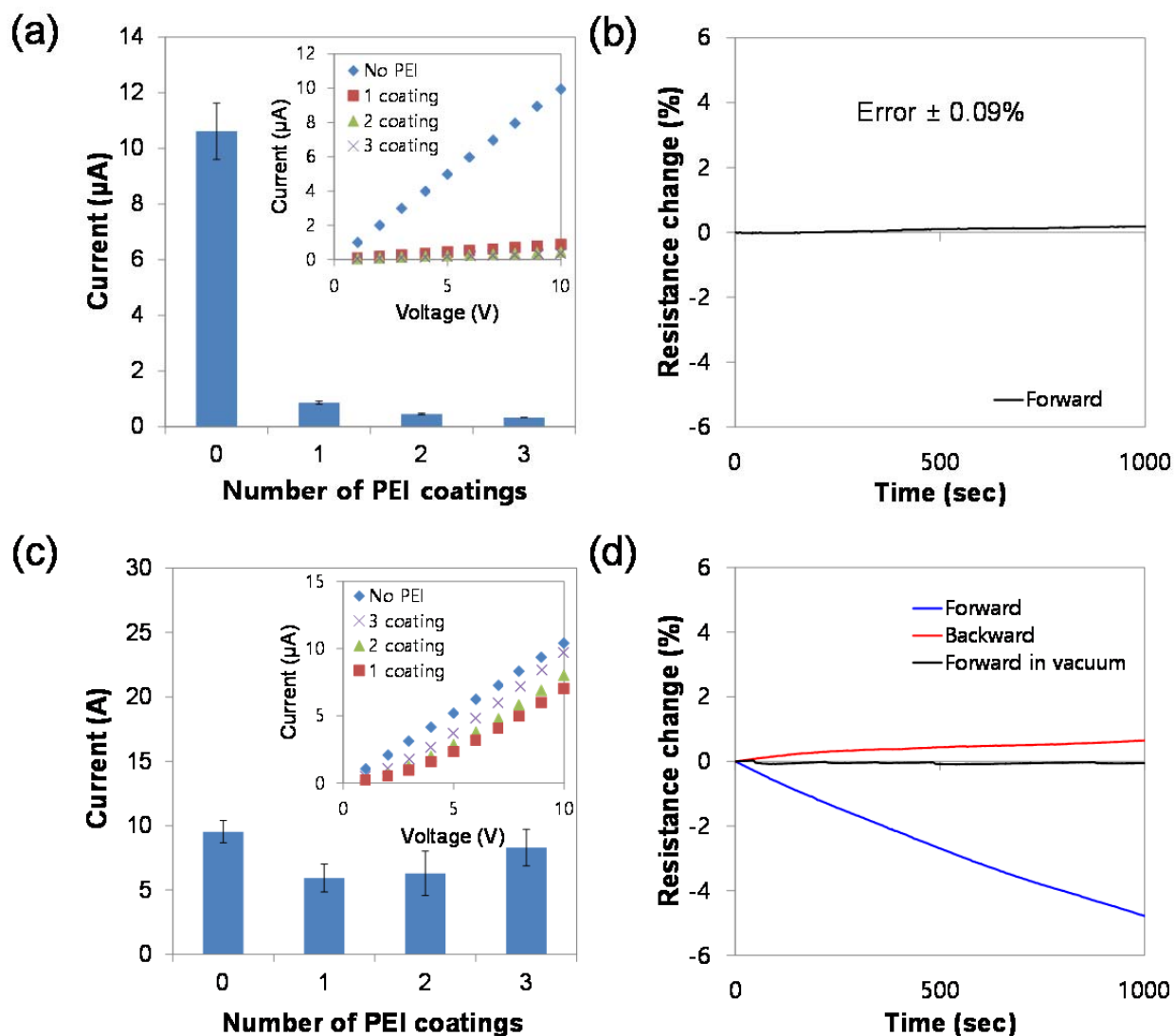


Figure 2.9. Resistance change for SWCNT/PEI and PEI/SWCNT lines (a) Current change at 10 V for 1, 2, and 3-PEI depositions. (b) Resistance change for an SWCNT/PEI line. (c) I - V characteristics of PEI/SWCNT lines for 1, 2, and 3-PEI depositions. (d) The resistance change of an SWCNT device for forward and backward printing directions in the air and forward direction in a vacuum (125 mmHg).

2.6 DISCUSSION

Due to the non-contact nature of the bridge-induced printing, the SWCNTs could be deposited without damaging substrate surface. The contact mode printing with the same capillary pen resulted in scratch marks on the substrate. The print line width was determined by the pen's outside diameter and the contact angle. For the examined ink formulation and substrate type, the contact angle was a function of substrate temperature and print speed. The smallest line width was obtained at $\theta_{B_a} \sim 90^\circ$ and was equal to the outside diameter of a pen nib. According to our characterization, PEI solution with known surface tension coefficient and viscosity showed that the line width was determined by solution viscosity at low speed (**Figure 2.10**). As the speed increased, the line width was determined by the contact angle and Capillary number. Since the flow rate decreased with the higher contact angle, the resulting sheet resistance increased at the lower flow rate. For example, using the smallest ink drop radius (110 μm) and water surface tension coefficient (0.073 N.m), the pressure difference (ΔP) at the substrate surface could range from 0 to 1.3 kPa. At room temperature, ΔP was close to 0 kPa due to the spreading of nano ink, which increased to 1.3 kPa at $\theta_{B_a} = 90^\circ$. Considering that the working principle yields a very low-pressure gradient in the system, the noncontact nano ink-bridge induced printing method can be beneficial for printing water-based molecular ink.

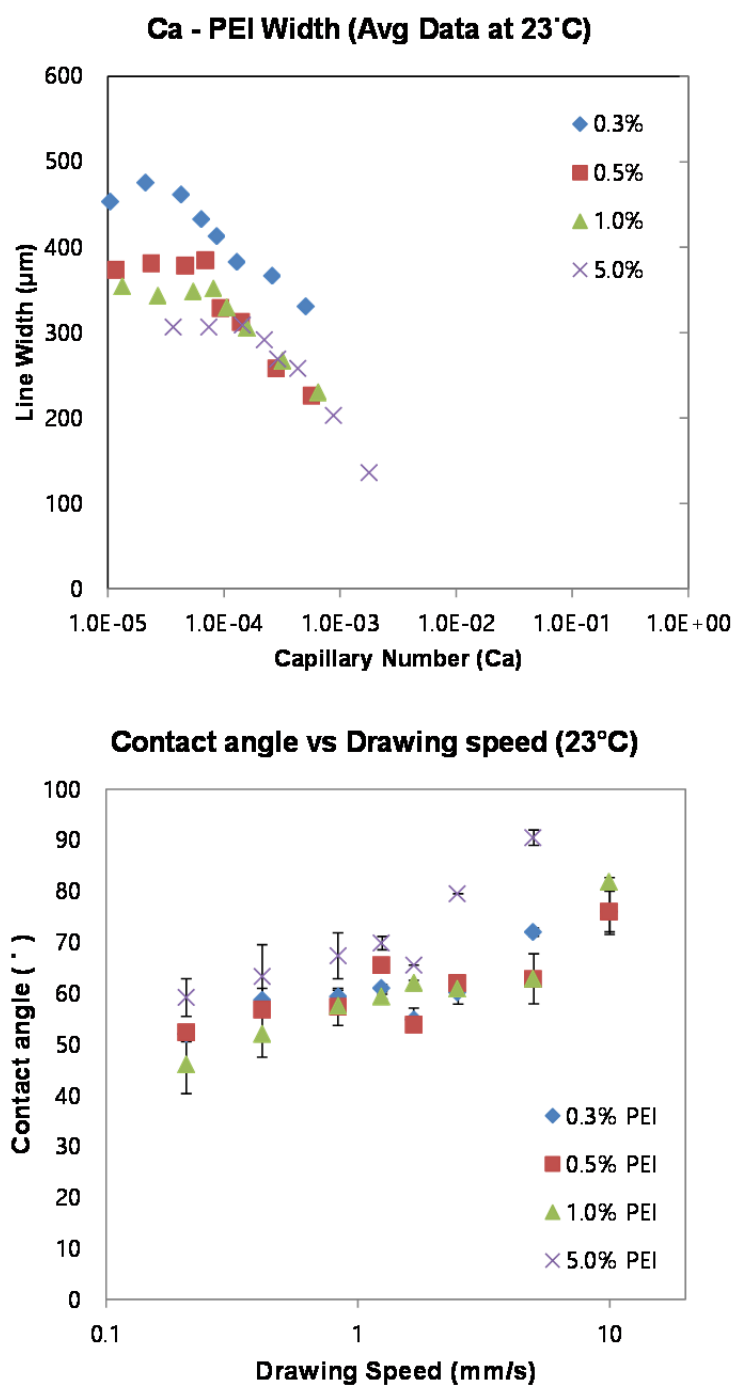


Figure 2.10. Line width for various concentrations of PEI diluted in deionized water.

2.7 CONCLUSION

In summary, the noncontact capillary pen printing method was developed for the fabrication of SWCNT sensors. Using a custom printer, the patterns of a dot, a line, and a film were printed and characterized in the contexts of morphology, electrical properties, and optical transparency. During the printing process, the contact angle was measured and related to the substrate temperatures and printing speeds. For a dot printing, a coffee ring effect was clearly shown for high-temperature substrate due to the rapid evaporation and the pinning effect in the ink bridge. The contact angle gradually decreased at room temperature, which formed a relatively uniform height of an SWCNT dot. For a line printing, the advancing contact increased as the substrate temperature, and the print speed increased. For these conditions, the higher pressure at the substrate reduced the ink flow rate increasing the sheet resistance and the optical transparency of the SWCNT line. For print uniformity, optimal printing temperatures are in the 60~80 °C range. A film could be printed to obtain an average sheet resistance of 7.2 k Ω /sq by a single printing at 60 °C. To obtain consistently high-quality prints, the advancing contact angle needs to be monitored. The nano ink bridge-induced printing allows for printing complex sensor geometries without damage of previous layers. The non-contact printing approach facilitates printing of large array sensors at low cost for wearable and film-type platforms.

Chapter 3. FABRICATION AND CHARACTERIZATION OF CHEMICAL SENSORS

3.1 INTRODUCTION

The significance of monitoring gaseous pollutants spans multiple areas vital to the environment and human health. The use of flexible and low-cost sensors in many applications has significant advantages over more expensive gas analyzers. These advantages are useful to sensor networks, where the area sample has to be taken at multiple locations, and personal exposure monitors, where the assessment of the exposure needs to be characterized from the perspective of the moving subject. Recent literature shows that exposure to gaseous pollutants can trigger diseases, including asthma and cardiovascular disease [87-89] and significant lung inflammation [90-96]. Various sensors have been developed in the last few decades, such as electrochemical detection, semiconductors, photoionization (PID), catalytic detection, and infrared detection. For individual use, lightweight gas sensors are fabricated by using field effect transistors (FETs). Heating to a few hundred degrees can improve the low sensitivity, but heating requires significant power (>100 mW). Typical sensitivity is at a sub-ppm level with limited specificity. Therefore, a portable and high-performance gas sensor is an urgent need for the assessment of individual exposure to toxic gases.

Recent advances in nanomaterial research have resulted in the development of novel sensor platforms [97-102]. Among nanomaterial gas sensors, carbon-based materials, including amorphous carbon, carbon nanotubes (CNTs), and graphene sheets, were very popular due to their well-developed synthesis methods and well-characterized detection mechanisms [103-106].

In comparison to graphene, SWCNTs show a higher sensitivity because their effective density of states around the Fermi level is lower than that of graphene [107]. The rolled structure of SWCNTs provides greater edge effects than that of the planar structure of graphene, which contributes to the superior sensing performance of SWCNTs. Since the first discovery of a CNT gas sensor [108], sensors for the detection of various gas molecules were developed (e.g., NO₂ [109] and NH₃ [110]). To enhance sensitivity, the CNT matrix needs to have high density with controlled doping levels of SWCNTs. To enhance specificity, an array of sensors can be used to mimic an olfactory system response [111], and a specific target can be identified by mapping the response of the array.

Further, an array of 32 SWCNT sensors were integrated into a silicon chip, which could identify NO₂ from humidity and chlorine [112]. However, the fabrication process (specifically, the precise functionalization of the SWCNTs) is challenging when micromachining is used. Simple dropping and drying of dopants on SWCNTs do not offer reliability in sensor fabrication or reproducibility in performance.

To overcome the drawbacks of micromachining-based processes, various bottom-up methods were developed. Early methods relied on direct growth using self-assembly aided by electric or magnetic fields [83, 84]. In order to print multiple layers of nanomaterials, various approaches have been investigated. Among them, non-contact printing is preferred to avoid potential damage to the substrate or the previously-printed layers. Inkjet-style printing [60-62] is the most common method of various commercially available techniques. Thermal and piezoelectric inkjet printing use thermal expansion or electromechanical vibration to eject droplets, overcoming surface tension in the formation of droplets [60, 63]. Due to the physics of droplet formation, inkjet printing is limited to the range of viscosities and values of surface

tension of the working fluid [64, 65]. When the nano-ink of interest has high surface tension and low viscosity, cumbersome change of ink properties is performed to generate a stable droplet [32, 66, 67]. In addition, CNTs could be easily clogged in the nozzle, and repeated writing is required to connect the printed dots. Polymer doping on SWCNTs was another challenge because of the discontinuous pattern. Microcontact printing was capable of patterning CNTs, but defects could be generated due to the nonuniform contact [113]. Fountain pens [76-78], ball pens [79], and pencils [80, 81] have been demonstrated in nanomaterials printing. However, these contact printing methods could induce damage to the substrate [78], which impeded the printing of multiple layers or large scales. A pencil made of carbon nanotubes could be used, but the substrate was limited to paper with a lack of doping capabilities [81].

In summary, despite the great potential of SWCNTs for the sensitive and selective sensing of gas molecules, current micromachining and printing methods do not meet the controlled doping and reliable printing needs to be required for SWCNTs. A reliable printing method is crucial for the development of a sensor array with enhanced sensitivity and specificity. Such challenges have limited the use of SWCNT sensors to resistive sensing mechanisms. SWCNTs have not been demonstrated for capacitive and inverter gas sensor use. To address these challenges, we will use a noncontact capillary pen to integrate SWCNT-based capacitance, resistance, and inverter sensors into a sensor array. Density functional theory-based computation will elucidate the sensing mechanisms in order to enhance the detection limit for various target gas molecules.

3.2 OBJECTIVES

Using the developed printing method in Chapter 2, a continuous SWCNT line will be patterned for SWCNT sensors. Polymers as activation material will be aligned and printed on the SWCNT line for doping. Using liquid bridge, PEI and nafion will be deposited without damaging previous patterned SWCNTs line. Ammonia and NO_x gas are tested.

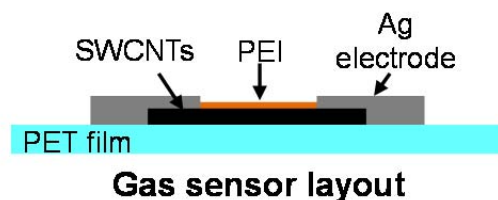
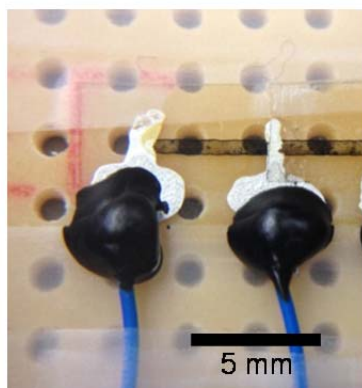
For pH sensor, two silver electrodes are stamped first, and then one of them is changed to AgCl as a reference electrode by electrolysis. The pH sensor performance will be characterized.

3.3 SENSOR FABRICATION

Gas sensor To fabricate a gas sensor, SWCNT electrode was printed as a line using a 300 μ m-diameter pen (**Figure 3.1a**). The substrate temperature was held at 80 °C, and printing speed was 0.83 mm/s. Silver ink was deposited on both ends of the SWCNTs for electrical connection. The SWCNT electrode was functionalized by depositing 1 μ l drop of 1 % PEI, which was cured for 1 hr at 100 °C in a convection oven. A second SWCNT electrode was coated with 1 μ l drop of 1 % Nafion (Nafion 117 solution, Sigma-Aldrich Co, LLC.) and cured for 1 hr at 120 °C in a convection oven. Both sensors were exposed to two different concentrations of NO₂ (8 and 22 ppm). The sensors' resistance

was measured and recorded by a multimeter (287 True RMS Multimeter, Fluke Corporation).

(a)



(b)

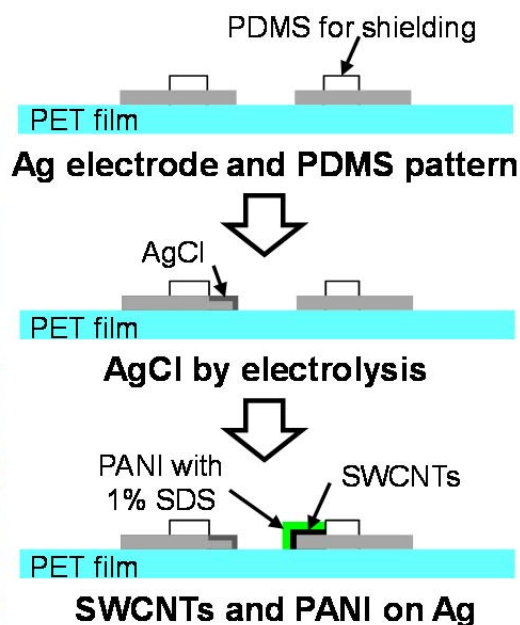
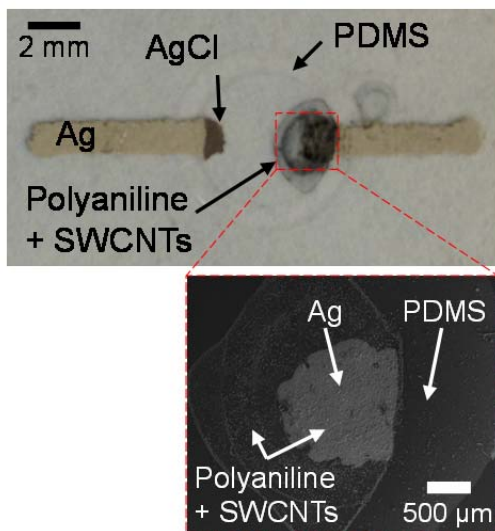


Figure 3.1. Fabrication steps (a) Images and cross-section of an SWCNT-gas sensor (b) Optical and SEM images and fabrication steps of an SWCNT-pH sensor.

pH sensor Silver electrodes were screen-printed on PET film (**Figure 3.1b** and **3.2**), which was cured at 100 °C for 10 min. Polydimethylsiloxane (PDMS; Sylgard 184 silicone elastomer, Dow Corning Corporation) was stamped in a ring shape to hold

analyte solution inside the ring. The PDMS was cured at 75 °C for 1 hour in a convection oven. One of the silver electrodes (cathode) was modified to form an AgCl layer by electrolysis (1.5 V_{dc} for 1 minute in 1 M HCl solution). The other electrode (anode) was printed with SWCNTs. The SWCNT (5 mg/ml) solution was deposited via capillary bridge printing using a 300 µm-diameter pen that covered the entire silver electrode surface. The SWCNT printed electrode was cured for 10 min on a 100 °C hotplate. Polyaniline (5 mg/mL; PANI; emeraldine salt, Sigma-Aldrich Co, LLC.) suspended in 1 % SDS was deposited on top of the SWCNT electrode using two 1µL drops, followed by curing at 120 °C for 1 hour. The voltage between the AgCl electrode and PANI electrode was measured using an Arduino circuit for standard pH solutions of pH 4, 7, and 10 (Omega Engineering, Inc.). Since the circuit measured the voltage difference ranging 0~3 V, a 1.61 V AA battery was serially connected to shift the voltage potential above 0 V in the control pH ranges (pH 7~10).

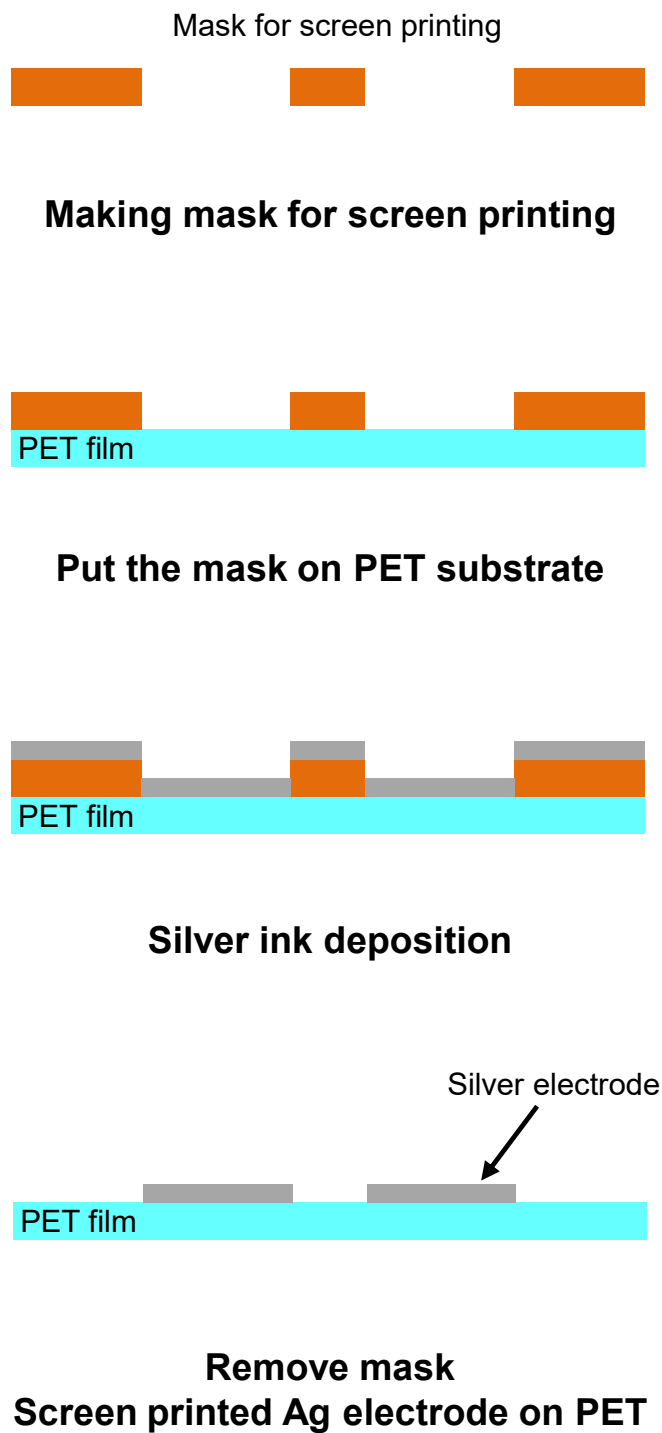


Figure 3.2. Silver electrode patterning. Sensor electrodes for a pH sensor are screen-printed to form silver electrodes on a PET film. The mask material for screen printing is PET film

3.4 EXPERIMENTAL RESULTS

Gas sensor The section aims to characterize an SWCNT gas sensor. When SWCNTs were doped with PEI and nafion, the sensor showed selectivity due to electrostatic interaction. PEI doped SWCNTs could selectively detect NO_2 while nafion doped SWCNTs could selectively detect NH_3 .

The non-contact printing technique was used for fabrication of a gas sensor. To achieve uniform resistance, PEI was coated on an SWCNT line. The SWCNT sensor doped with PEI showed a response to NO_2 gas (**Figure 3.3a**). Nafion-doped SWCNTs showed a negligible change when exposed to NO_2 gas (**Figure 3.3b**) but had a significant response to ammonia (**Figure 3.4**). The specificity of the SWCNT-sensors to NO_2 and ammonia gases were consistent with the previous report [110]. However, the response trends were opposite from the reported trends using n-type SWCNTs. The doping can change the sensitivity and selectivity of an SWCNT sensor [114]. For example, a positively charged dopant makes an SWCNT sensor sensitive to negatively charged gas molecules but insensitive to positively charged gas molecules. Without doping, an SWCNT sensor is also sensitive to gas molecules regardless of the electric charge of gas molecules. Note that our results were from SWCNTs printed on a PET film while the previous report used semiconducting SWCNTs grown on the micromachined electrodes.

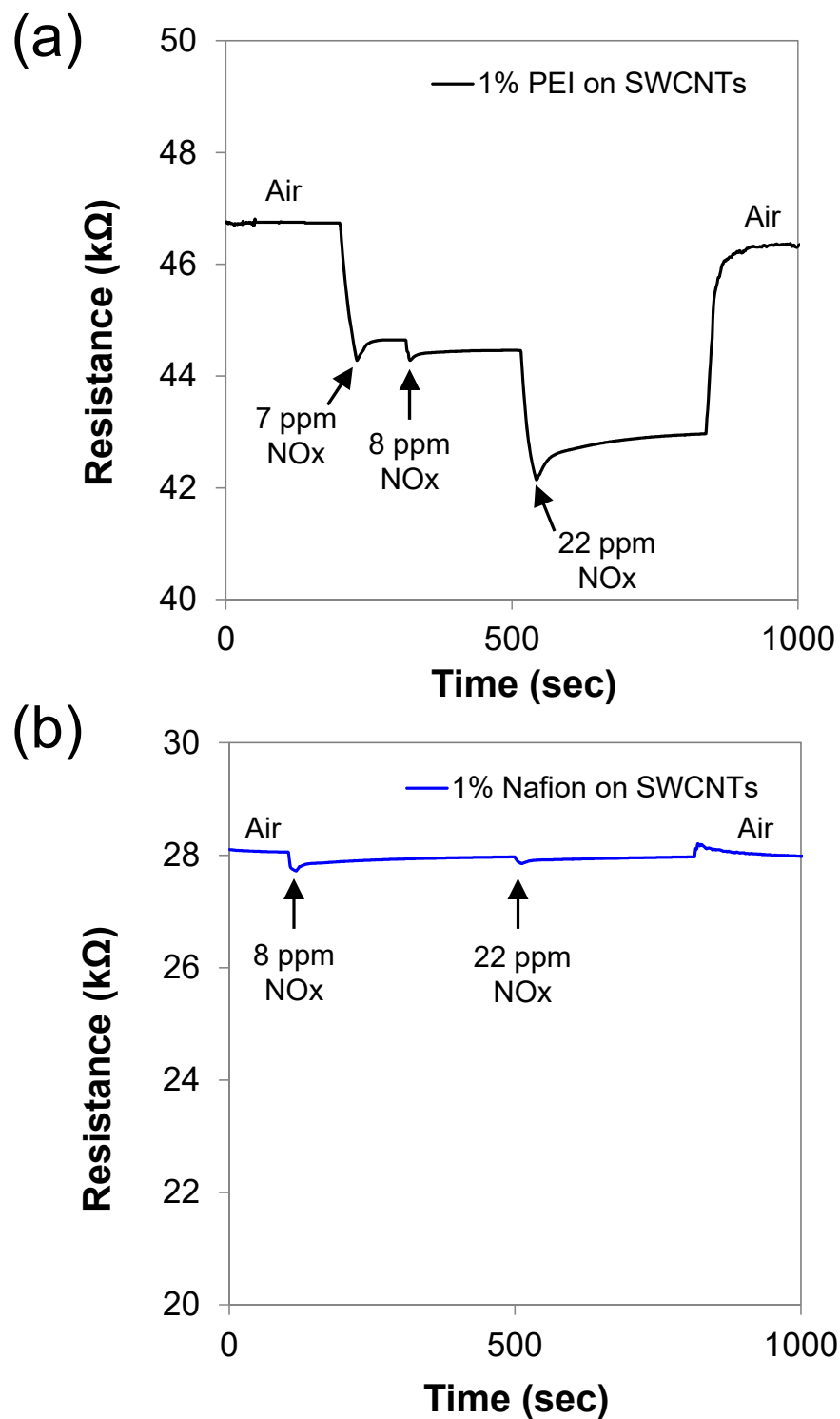


Figure 3.3. Gas response test (a) Change of PEI-doped SWCNT resistance for NO_x gas, (b) Change of Nafion-doped SWCNT resistance for NO_x gas.

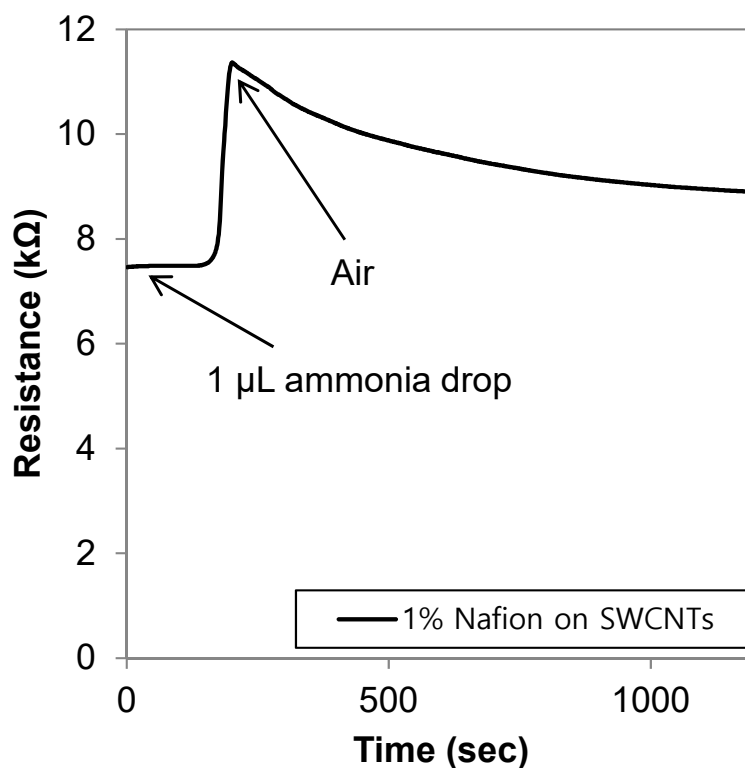


Figure 3.4. Change of Nafion-doped SWCNT resistance for ammonia gas. Nafion can differentiate between NO_x and Ammonia with high accuracy, showing specificity.

For ammonia detection, SWCNT sensors with and without Nafion doping were prepared. A commercial sensor was also prepared to calibrate the concentration of ammonia. The ammonia concentration was changed from 1 to 20, 120, and 2580 ppb, the resistances of the SWCNT sensors increased (**Figure 3.5a**). At 1 ppb, the resistance did not change. As the ammonia concentration increased, the resistance increased with different sensitivity for the SWCNT sensors with and without Nafion doping. The commercial sensor showed a response from 120 ppb to 2580 ppb, which showed lower sensitivity than the SWCNT sensors. Since the sulfonic group in Nafion was negative, the Nafion-doped sensor was sensitive to ammonia but

did not show the response to NO_2 . (**Figure 3.5b**) shows the sensitivity of the SWCNT sensors to ammonia. Considering the electrical noise level, the detection limit is between 5 and 10 ppb.

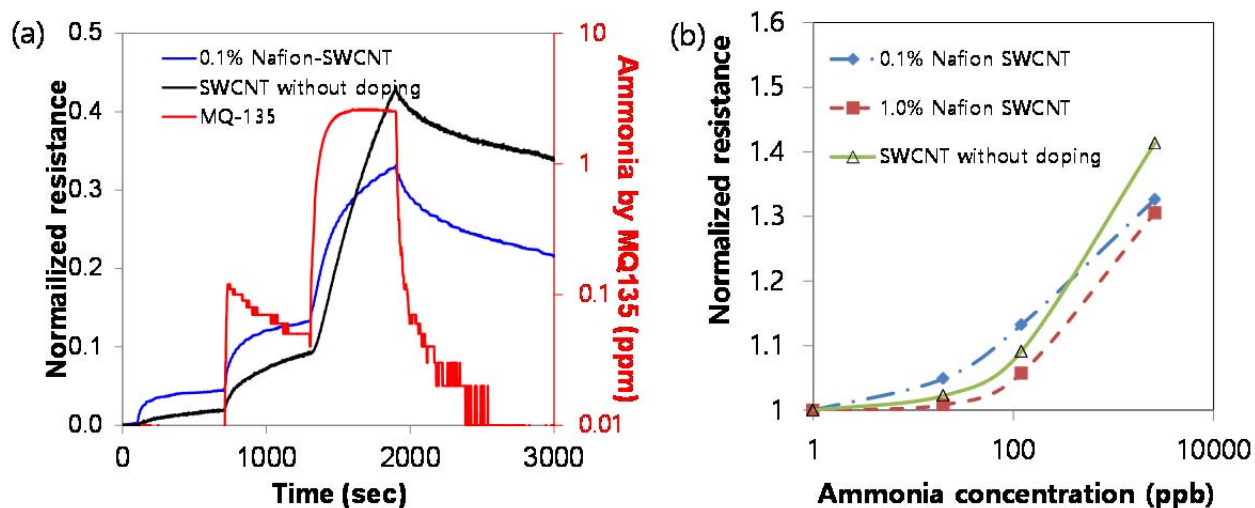


Figure 3.5. (a) Response of 0.1% Nafion-doped SWCNT, non-doped SWCNT and MQ-135 (commercial) sensors for ammonia concentrations of 1, 20, 120 and 2,580 ppb. (b) The sensitivity of SWCNT sensors with 0.1% Nafion and 1% Nafion doping and without doping. Normalized resistance change vs. ammonia concentrations.

pH sensor The section aims to characterize an SWCNT-pH sensor. The sensitivity showed 61 mV/pH. A pH sensor was fabricated using silver electrodes (**Figure 3.6**). As for the fabrication, silver electrodes were stamped on a PET film. One of the silver electrodes (cathode) was modified to form an AgCl layer by electrolysis (1.5 V dc for 1 minute in 1M HCl solution). The other electrode (anode) was printed with single-wall CNTs (SWCNTs) using the capillary pen printing method. The SWCNTs were sensitive to pH and enhance electron transfer on the electrode surface. The SWCNT solution (5 mg/ml) was deposited via capillary bridge printing using a 300 μm -diameter pen that covered the entire silver electrode surface. The SWCNT

printed electrode was cured for 10 min on a 100 °C hotplate. Polyaniline (5 mg/mL; PANI; emeraldine salt, Sigma-Aldrich Co, LLC.) suspended in 1 % SDS was deposited on top of the SWCNT electrode using two 1 μ L drops, followed by curing at 120 °C for 1 hour.

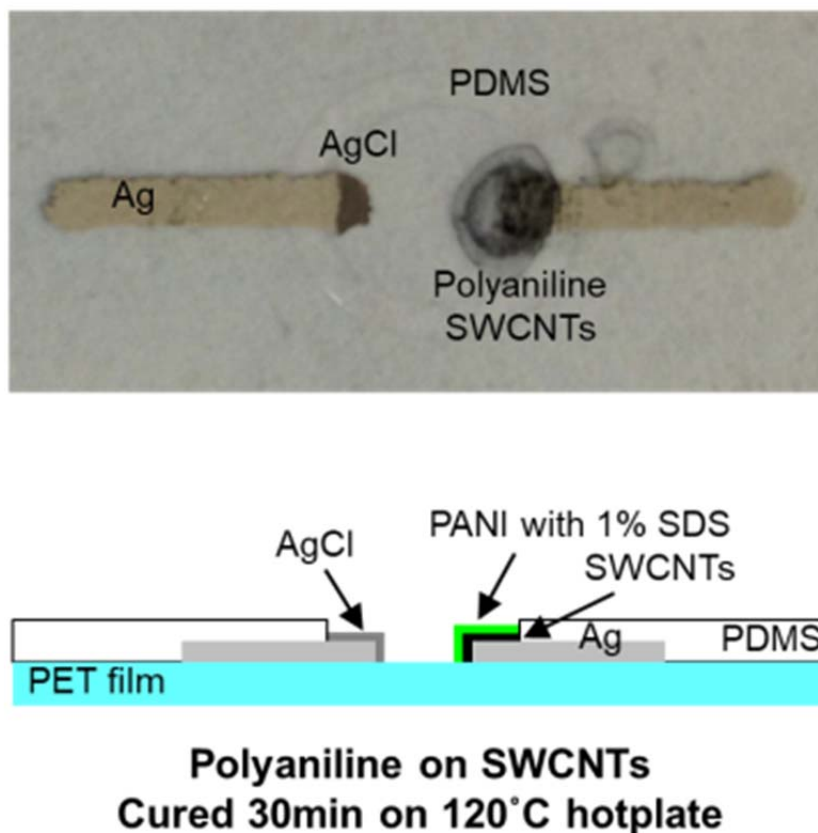


Figure 3.6. Fabricated pH sensor

The voltage between the AgCl electrode and PANI electrode was measured using an Arduino circuit for standard pH solutions of pH 4, 7, and 10 (Omega Engineering, Inc.) (**Figure 3.7a**). Since the circuit measured the voltage difference ranging 0~5 V, a 1.61 V AA battery was serially connected to shift the voltage potential above 0 V in the control pH ranges (pH 4~10).

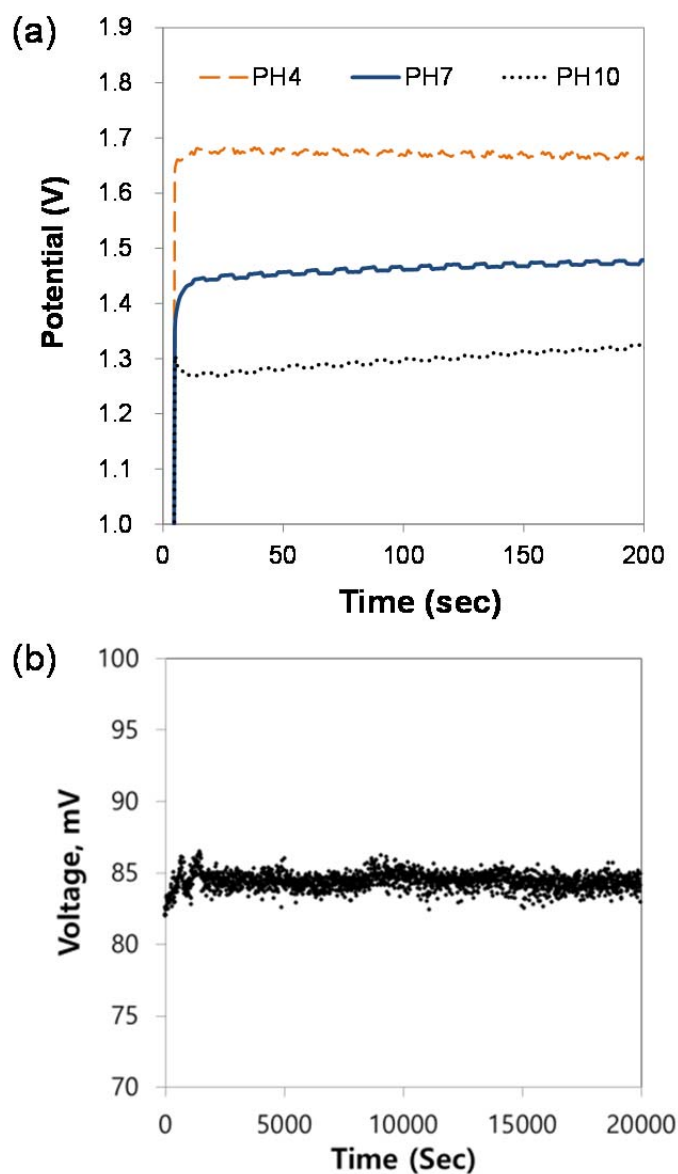


Figure 3.7. (a) Voltage measured for a pH sensor using standard solutions of pH 4, 7, and 10. Note that the voltage is shifted by using a 1.61 V-AA battery. (b) Stability of a pH sensor for 20,000 seconds

When the solution drops of pH = 4, 7, and 10 were sequentially interrogated using the fabricated sensor, the potential decreased as pH changed from pH 4 to 10. Note that the actual potential needs to be subtracted from the measured value because a 1.61 V bias potential was

added to the circuit. The average slope was 61 mV/pH at 100 s, which was consistent with the theoretical Nernstian slope as mentioned in equation (3). When NaCl salt was added to the standard pH4 solution, the potential becomes stable as 84 mV for 20,000 seconds (5.5 hours) with temperature calibration (**Figure 3.7b**).

3.5 DISCUSSION

Using the SWCNT sensor, pH was measured for pH 4, 7, and 10 solutions. The voltage generated from the electrode surface was dependent on ion concentration, which is described by the Nernst equation.

$$E = E^0 + 2.303 \frac{RT}{nF} \log_{10} \left(\frac{[Ox]}{[Red]} \right)$$

, where Ox: oxidized species, Red: reduced species, []: molar concentration, F : Faraday constant (96485 C/mol), R : gas constant (8.314 J/K mol), n : The number of electrons transferred. E^0 : Potential in comparison to hydrogen electrode. T : absolute temperature.

When silver chloride electrodes were used, the reduced species becomes 1; the equation is simplified into $E = E^0 + 0.000198T \log_{10}[Ox]$. When the temperature was room temperature (298K), $E = E^0 + 0.0591 \log_{10}[Ox]$. Therefore, the potential change becomes 59.1 mV at room temperature when the concentration of a target ion changed by 1 order of magnitude. In our

results, 61 mV/pH was measured in average for the standard pH solutions, which agreed with the theoretical value.

The major difference between conventional ion sensors and the fabricated sensor was in the solid-state layers for pH measurement. Conventional ion sensors are bulky with solution-phase transfer mechanism of ions through an ion selective membrane. The fabricated sensor used SWCNTs as a transducer to change pH into voltage potential. For selective detection of pH, ion selective membrane needs to be added onto the pH sensor.

3.6 CONCLUSIONS

In summary, a gas sensor and a pH sensor were demonstrated using the SWCNT sensors printed with the capillary pen method. The SWCNT sensor doped with PEI showed a response to NO_2 gas. Nafion-doped SWCNTs showed a negligible change when exposed to NO_2 gas but showed very high sensitivity to ammonia gas (3 ppb). The pH sensor showed the voltage potential of 61 mV/pH, which was close to the theoretical value of 59.1 mV/pH.

Chapter 4. A FLEXIBLE IMMUNO-SENSING PLATFORM USING SINGLE-WALLED CARBON NANOTUBES (SWCNT)

4.1 INTRODUCTION

Tuberculosis, caused by *Mycobacterium tuberculosis* (MTB), is one of the serious infectious diseases worldwide. Although the case number gradually declines, developing countries have shown a significantly higher mortality rate[115]. In Asian and African countries, over 80% of populations show positive cases[116]. For initial TB screening, three sputum samples are collected from a patient in the early morning [117]. The sample collection procedure is repeated for a few times in order for initial screening. The collected samples are diagnosed with various methods, such as a Ziehl-Neelsen (ZN) method for microscopic detection, immunoassays for antigen detection[118], polymerase chain reaction (PCR) for DNA or RNA detection[119, 120]. The ZN smear method is labor-intensive, and not sufficiently sensitive for TB diagnosis. Enzyme-linked immunosorbent assay (ELISA) is a rapid and relatively easy tool but with low sensitivity. Among the approaches, PCR-based methods have shown clinical sensitivity and specificity greater than 95% but with 2 hours of detection time [121].

In the whole screening time, the significant time for the screening is occupied by the liquefaction process of sputum samples. Since sputum samples are thick and viscous, the samples need to be dissipated potentially with disinfection process for safe screening. The processing time typically requires at least 20 minutes, which has been a major hurdle for point-

of-care (POC) screening of TB. In addition, sputum sample collection has been a challenging problem for kids and babies. Recently, a clinical study has been conducted to discover a new biosample source. Oral swab samples have been found as a convenient and noninvasive source for TB diagnosis [122, 123]. Using oral swab samples, a POC screening tool can be realized as far as a simple but highly sensitive and specific sensor is offered.

For a highly sensitive and specific screening tool, nanomaterials have been persistently investigated for infectious disease diagnosis. Among the nanomaterials, single-walled carbon nanotubes (SWCNTs) are one of the potential candidates to enable a simple resistive transducer to detect the binding of a target analyte with high sensitivity. In comparison to optical and fluorescent detection, a resistive sensor runs with small power and small form factors. Since optical focus and magnification is not required, a light pocket-size device can be developed as a measurement unit. The high sensitivity of an SWCNT biosensor is originated from the small diameter (~ 1 nm) comparable to the size of single biomolecules and the thickness of electrical double layers in physiological buffer [124].

Resistive SWCNT sensors can detect targets by two distinct mechanisms [125-128]. One is to change the free carrier density of doped SWCNTs by electrostatic interaction. The other is to change the work function of the metal electrodes-SWCNT interface, thus leading to Schottky barrier modulation. For semiconducting nanotubes, both mechanisms play a role in modulating the resistance. When SWCNTs were deposited on gold electrodes patterned on a silicon substrate, viral particles and bacteria could be detected by measuring the resistance change [129, 130]. The detection limit of swine influenza virus (H1N1) was 177 TCID (50 % Tissue culture Infective Dose) /mL [129]. The detection limit for *B. Subtilis* was 100 CFU/mL [130]. SWCNTs functionalized with heparin could detect dengue virus as low as 840 TCID/mL [131]. The lowest

detection limit was 1 PFU/mL to detect H1N1 [132]. In addition, a similar configuration of SWCNT sensor was applied to detect peanut allergen in food extract with the detection limit of 5 ng/mL [133]. In mRNA detection, the detection limit was demonstrated in an attomolar level, which has the potential to detect nucleic acid without amplification [134]. In our previous work, nanotips made of SWCNTs could be used for bacterial detection [135]. The crossbar junctions coated with SWCNTs were fabricated to detect target bacteria in food samples [136]. The sensor substrate, electrical layers, target samples, detection limit, and targets are summarized in **Table 2**.

Number	reference	Substrate	Electrical layer	Target sample	Detection limit	Target
1	[132]	Silicon/oxide	On Au	Buffer	1 PFU/mL	H1N1
2	[130]	Silicon/oxide	On Au	Buffer	100 CFU/mL	B. Subtilis
3	[133]	Silicon/oxide	On Au	Food extract	5ng/mL	Ara h 6
4	[129]	Silicon/oxide	On Au	buffer	177 TCID/mL	H1N1 (10, 20 and 50 um gaps)
5	[131]	Silicon/oxide	On Au	buffer	840 TCID/mL	dengue virus
6	[134]	Silicon	On Au	buffer	aM	mRNA
7	[135]	Tungsten wire coated with Au	On Au	buffer	10 ³ CFU/mL	BCG bacteria
8	[136]	Crossing tungsten wires coated with Au	On Au	buffer	10 ² CFU/mL	Escherichia coli

Table 2. Resistive single-walled carbon nanotubes for detection of bioanalytes

In spite of the great potential as a POC screening sensor, few resistive SWCNT biosensors have been demonstrated for target detection in human samples. One of the main challenges was the screening effect by high ion concentrations in physiological buffer. Positive and negative ions covering SWCNTs rendered both electrostatic and Schottky modulation

disabled and unpredictable. For immunoassay, the challenge was resolved by rinsing with deionized (DI) water. The sensing mechanism of DI-rinsed SWCNTs could be recovered without losing the binding activity of antigen-antibody. In addition to the screening effect, hydrogen binding on SWCNTs in the water-based buffer could generate unreliable resistance change due to absorption and desorption of hydrogen. For example, when SWCNTs were bound with antibodies in the physiological buffer, hydrogen was bound on SWCNTs to increase the resistance. As soon as the sensor was exposed to air out of the buffer, the electrical resistance started to decrease. The resistance change by target bindings could be interfered with that by hydrogen release, which could reduce the sensitivity and reliability.

In this paper, a resistive SWCNT biosensor is fabricated on a flexible film, polyethylene terephthalate (PET) for low-cost TB screening. Silver electrodes are stamped as probing electrodes for SWCNTs. The sensing mechanism of SWCNTs coupled with silver electrodes is investigated in conjunction with hydrogen desorption. The sensitivity and specificity are characterized by MTB and surface antigen (MPT64) in PBS. Subsequently, the sensor is characterized by tongue swab samples spiked MTB and MPT64. Simple resistive measurement is conducted before and after immunocomplex formation for detecting targets. The presented biosensor will move one step further to a POC platform for rapid and low-cost TB screening.

4.2 OBJECTIVES

The objective in this chapter is to develop a flexible immuno-sensing platform using single-walled carbon nanotubes (SWCNT) with high sensitivity. A resistive SWCNT biosensor is fabricated on a flexible film, polyethylene terephthalate (PET) for low-cost TB screening.

Silver electrodes are stamped as probing electrodes for SWCNTs. The sensing mechanism of SWCNTs coupled with silver electrodes is investigated in conjunction with hydrogen desorption. The sensitivity and specificity are characterized by MTB and surface antigen (MPT64) in PBS. Subsequently, the sensor is characterized by tongue swab samples spiked MTB and MPT64. Simple resistive measurement is conducted before and after immunocomplex formation for detecting targets. The presented biosensor will facilitate a POC TB screening platform with a small form factor, low power requirement, and easy use.

4.3 EXPERIMENTAL METHOD

4.3.1 *Sensor fabrication*

The sensor was fabricated on a polyethylene terephthalate (PET) film (**Figure 4.1a**). Target cells and antigen are detected by using an SWCNT sensor functionalized with Polyethyleneimine (PEI) and antibodies (**Figure 4.1b**). Silver electrodes are patterned for resistive detection. When targets are bound on the sensor surface, the resistance decreases due to the electrostatic interaction.

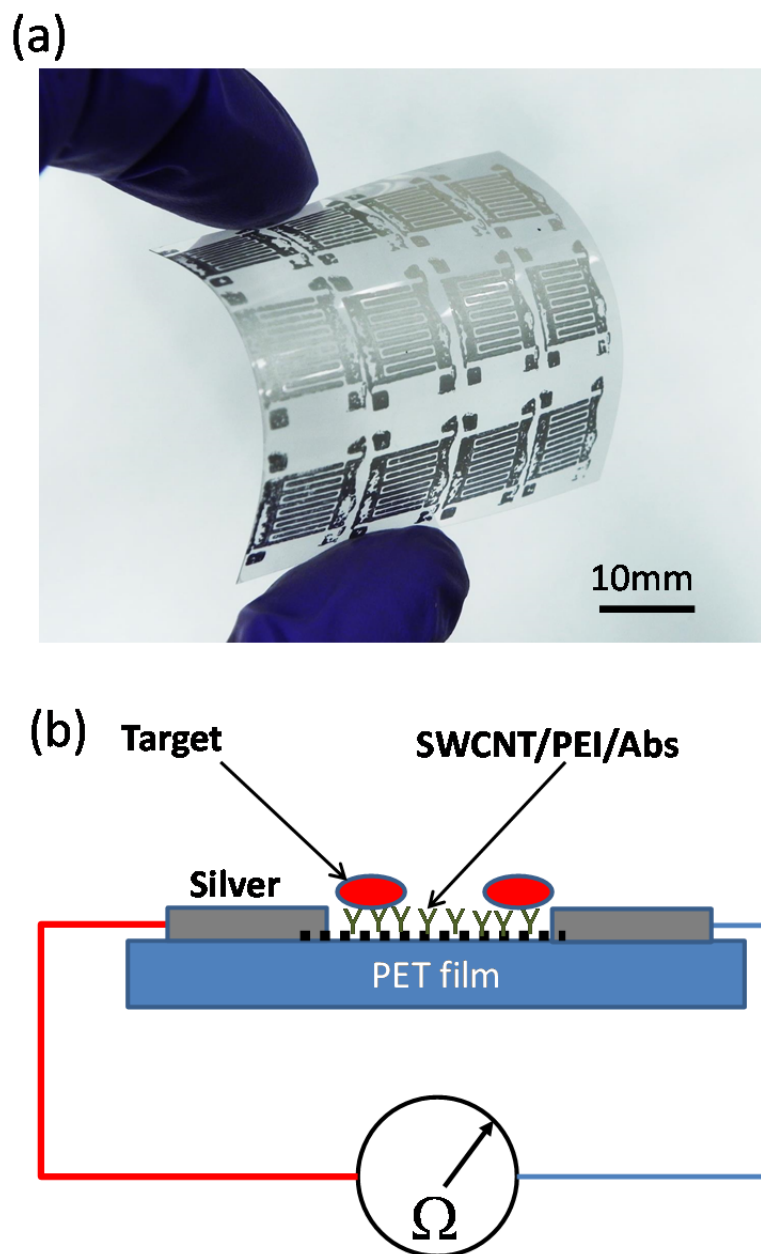


Figure 4.1. (a) An SWCNT-based sensor on a flexible PET film. (b) Cross section of a resistive SWCNT immunosensor.

For fabrication (**Figure 4.2a~4.2d**), SWCNTs were dispersed in SDS at a concentration of 1 mg/mL using a sonicator at room temperature for 8 hours. The SWCNTs were spin-coated on a

PET film at 6000 rpm for 20 seconds. PEI (0.1 %) was coated on the SWCNT surface. Silver ink was stamped on the PEI coated SWCNT surface. For silver electrode patterning, a Delrin® mold was machined by using an end mill. The stamp was made of polydimethylsiloxane (PDMS) cured in a mold at room temperature for 3 days. The PDMS stamp coated with silver ink was stamped on a PEI-coated SWCNT sensor. The sensor was cured on a hot plate for 10 min at 100 °C. A polyclonal IgY antibody (1.8 mg/mL) raised against MPT64 protein was physisorbed on the SWCNT surface for 24 hours in the refrigerator (4 °C). Subsequently, one sensor was cut by scissors by half in order to make 2 sensors. With the cutting, a total of 24 sensors could be fabricated on a 40x40 mm² PET film. **Figure 4.2e** shows a photo showing a sensor composed of two interdigitated electrodes. The gap is filled with functionalized SWCNTs. The magnified image shows the gap size of 200~300 µm.

Unlike other SWCNT immunosensors, the SWCNTs in a sensor were coated with silver electrodes, which minimized the exposure of the interfacial area between SWCNTs and silver electrodes. The sealing of the interfacial area could offer a uniform contact resistance and isolate the Schottky effect in the sensing mechanism. The electrostatic gating effect was only the mechanism to detect the target analytes. In the configuration, the oxidation of the silver electrode surface should not interfere with the resistive change for target detection.

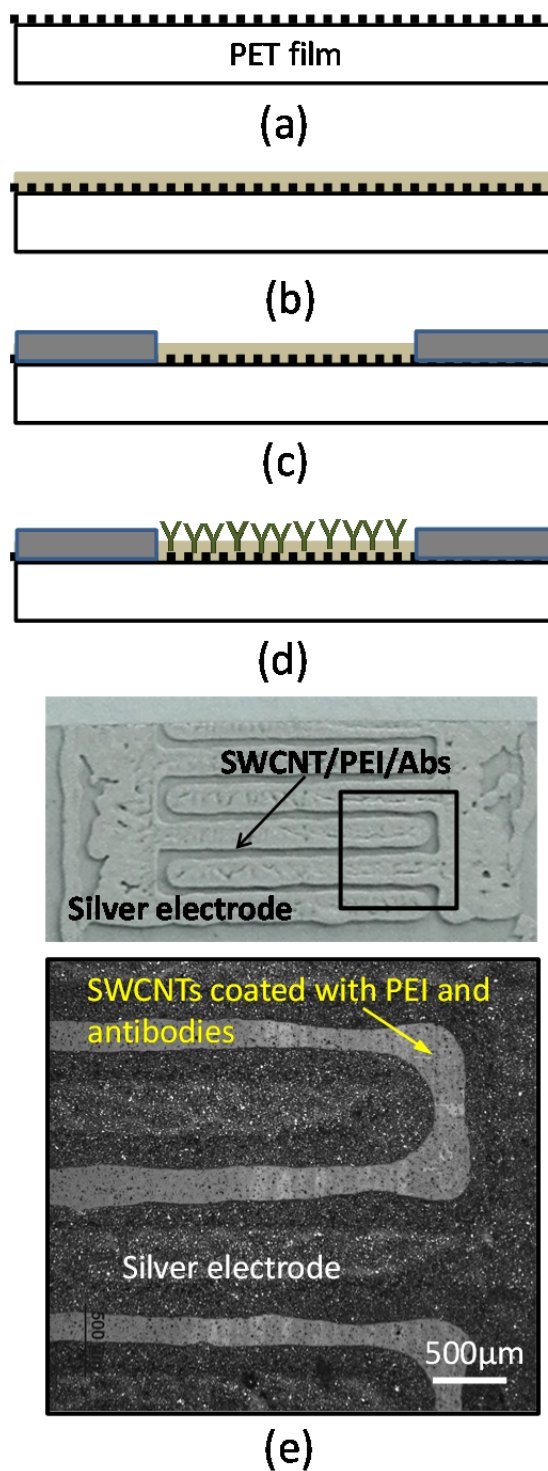


Figure 4.2. The fabrication process of an SWCNT-based immunosensor (a) Spin coating of SWCNTs on a PET film (b) Spin coating of PEI (c) Stamping of silver electrodes. (d) Antibody immobilization (e) Photo and optical microscope images of an SWCNT immunosensor.

4.3.2 Antibody preparation

A polyclonal IgY antibody (pAb) was raised against purified MPT64 protein in Aves Labs (Davis, CA, USA). Complete Freund's adjuvant was used; thus, antibodies were reactive to MTB as well as MPT64. The antibodies were raised in two hens and evaluated by ELISA to determine protein binding, and filter plate EIA to determine reactivity to target cells.

To evaluate antibodies against *Mycobacterium*, cultures of BCG and MTB H37Ra cells were diluted to 1×10^7 cells/mL in PBS. Cell solutions were then added to a 96-well filter bottom plate (Millipore 0.45 μ M, #MAHVN4510), 100 μ L of a 1×10^7 cells/mL solution of MTB or BCG. The cells were trapped by the 0.45-micron filter, and buffer/media was washed. Cells were washed with vacuum filtration, 3x200 μ L DPBS. 28 μ g/mL IgY antibody (100 μ L) was added to each well and incubated for 30 min at 37 °C. Control (pre-immune IgY) antibodies were tested at the same concentration. The IgY solution was washed with 4x200 μ L DPBS. A 100 μ L solution of a 2° antibody (1:1000 dilution Rab anti-IgY-HRP Conjugate, Thermo Scientific #31401) was added and incubated for 30 min at 37 °C, followed by DPBS washing (3x200 μ L). Finally, 100 μ L ABTS substrate (Thermo Scientific #37615) was added, followed by a 10-minute incubation at room temperature. The solution was then filtered through the filter plate and into a clear 96-well plate and read at A_{405} in a microplate reader. **Figure 4.3a** shows the positive results of the antibodies for both BCG and MTB H37Ra. According to the results, the polyclonal antibodies were specific to *Mycobacterium* strains, potentially, including non-tuberculosis *Mycobacterium* (NTM) species. The specificity test will be conducted using an SWCNT immunosensor.

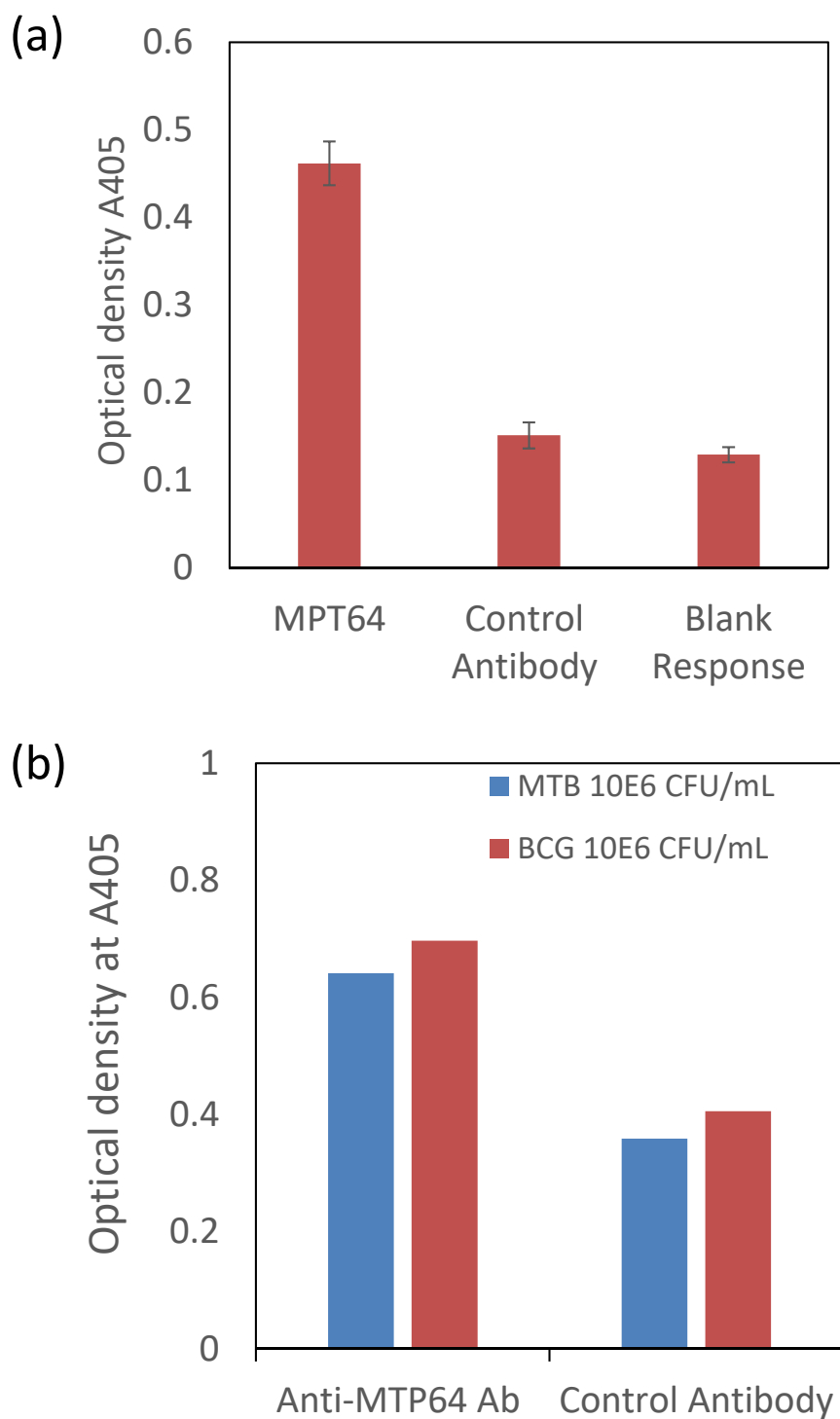


Figure 4.3. (a) Optical density showing the binding of MPT64 antibodies to MTB (10^6 CFU/mL) and BCG (10^6 CFU/mL) at $28 \mu\text{g/mL}$. (b) Optical density showing the binding of MPT64 antibodies to MPT 64 in comparison to control.

To assay the MPT 64 protein, 100 μL of a 100 $\mu\text{g/mL}$ solution of MPT-64 in DPBS was added to an ELISA protein binding 96-well plate (Immulon 2HB, Thermo Scientific #3455). The mixed solution was incubated overnight at room temperature, followed by 1 hour at 37 $^{\circ}\text{C}$, and then washed with 3x200 μL DPBS. To block the remaining sites in the well, a 200 μl BSA solution in DPBS at 1 mg/ml was added and incubated for 1 hour at 37 $^{\circ}\text{C}$ followed by washing with 3x200 μL DPBS. A 100 μL solution of IgY raised to MPT-64 was added to each well at a concentration of 28 $\mu\text{g/ml}$ in DPBS. Control (pre-immune IgY) antibodies were tested at the same concentration. A 100 μl solution of a 1:1000 Dilution of 2 $^{\circ}$ (Rab anti-IgY-HRP Conjugate, Thermo Scientific #31401) was then added and incubated for 30 min at 37 $^{\circ}\text{C}$, followed by DPBS washing (3x200 μL). Finally, 100 μl of ABTS substrate (Thermo Scientific #37615) was added and measured at A_{405} after 10-minute incubation at room temperature. **Figure 4.3b** shows the positive results from the antibodies to MPT-64 compared to pre-immune antibodies.

It is acknowledged that antibody secretion and characterization were conducted by Mr. Soelberg in Furlong's group. We appreciate the advice and support for the successful development of the TB screening assay.

4.3.3 *Sensor characterization*

After antibody immobilization, the resistance of SWCNT sensors increased due to antibody binding and hydrogen binding on SWCNTs. To study the contribution of resistance increase in an antibody immobilization step, the antibody concentration varied from 0, 0.9, 1.8, and 4.5 mg/mL in PBS buffer. After 24 hours of incubation of SWCNT sensors in each solution, the normalized resistance change was measured before and after antibody immobilization.

As soon as the sensor was exposed to air, the sensor resistance started to decrease due to the desorption of hydrogen. The desorption process was critical to obtain a reproducible resistance measurement after binding targets. To characterize the resistance change due to the hydrogen desorption, the resistance change was measured for 5 hours. One sensor was incubated at 25 °C, and the other sensor was incubated at 35 °C on a hot plate. The relative humidity was 25~30 % in the tests. Since antibodies were immobilized on the sensor surface, the sensor temperature was not tested over 35 °C in order to avoid potential damage or degradation of antibodies.

To characterize how the sensor response was changed due to hydrogen desorption, the sensor response to targets (MTB at 10^6 CFU/mL in PBS) binding was tested after 5, 20, 40 and 120 minutes of incubation at both 25 and 35 °C. The sensor response for MTB at 10^6 CFU/mL in PBS was compared for each condition in comparison to the control (PBS). The incubation time of 5, 20, 40, and 120 minutes was determined in consideration of the slope change of the resistance at 35 °C.

4.3.4 *Sensitivity and specificity tests*

For sensitivity and specificity tests, both MTB and MPT64 were suspended in 1x PBS buffer. For MTB, various concentrations of MTB cells were suspended in PBS from 10^1 ~ 10^5 CFU/mL. MPT64 was also suspended in PBS from 0.1 ng/mL to 1 µg/mL with 10 fold dilutions. 1 mL of each solution was supplied in each plastic cup where a sensor was dipped for immunocomplex

formation. After 10 min of incubation, the sensor was rinsed with DI water. After the gentle blow dry with nitrogen, the resistance was measured. The resistance values before and after immunocomplex formation were R_0 and R_f , respectively. The normalized resistance change $[(R_f - R_0)/R_0]$ was computed to compare the normalized resistance change from control.

For specificity test, the response for MTB (10^2 CFU/mL) was compared with *Staphylococcus epidermidis* (S. Epi at 10^3 CFU/mL), *Mycobacterium Avium* (M. Avium at 10^3 CFU/mL), and *Mycobacterium Bacillus Calmette–Guérin* (M. BCG at 10^3 CFU/mL). Each bacterium species was suspended in 1 mL-PBS.

4.3.5 *Test using tongue swab samples*

As described earlier, a tongue swab sample was one of the new types of oral swab samples that contained MTB markers. To evaluate LLD for MTB and MPT 64 in tongue swab samples, swab samples were prepared by scraping tongue surface from deidentified volunteers as illustrated in **Figure 4.4**. After a complete dry of swabs in air, the swab samples were immersed in 1 mL PBS contained in an individual microtube for 20 minutes with gentle stirring. Subsequently, 500 μ L of target analytes (MTB or MPT 64) in PBS was mixed with 500 μ L of the eluted swab solution. The 1 mL solution was used to test LLD. The concentrations of MTB were $10 \sim 10^5$ CFU/mL with 10 fold dilutions. The concentrations of MPT64 ranged from 1 ng/mL to 10 μ g/mL with 10 fold dilutions. A sensor was incubated in 1 mL sample solutions for 10 minutes. After rinsing with DI water, the resistances were measured. Before and after the incubation, the resistances were measured to compute a normalized resistance.

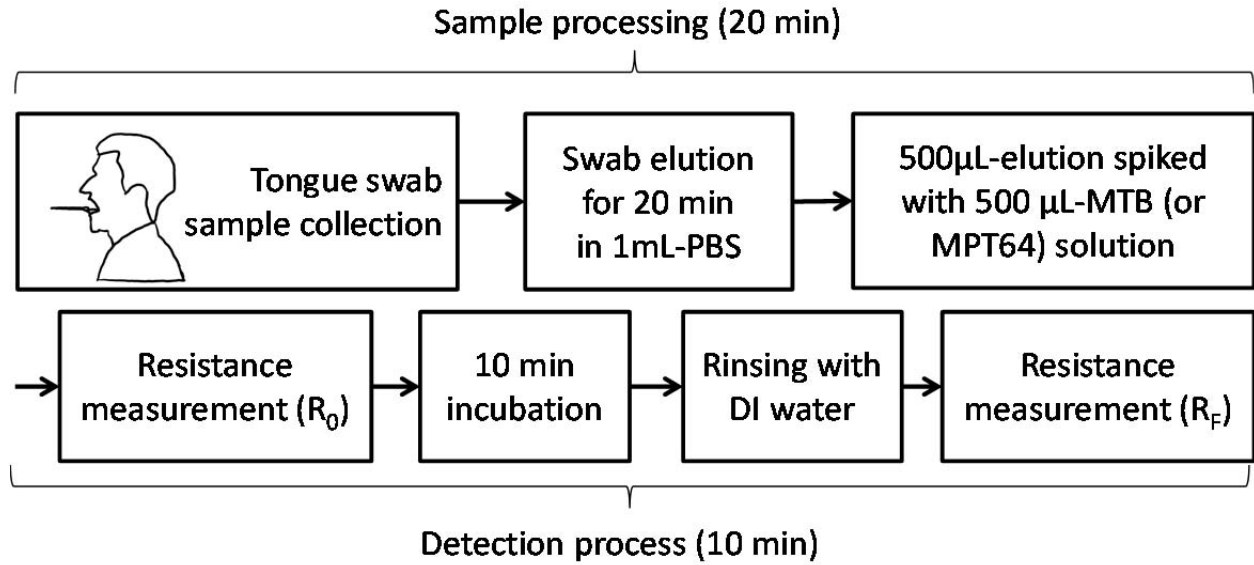


Figure 4.4. Preparation protocol of tongue swab samples and resistive detection procedure.

4.4 EXPERIMENTAL RESULTS

In the antibody immobilization step of 24 hours, the resistance of SWCNT sensors was changed by the bindings of both hydrogen and antibodies. Since the most ions in PBS were washed in the rinsing step after antibody immobilization binding, the effect of ions in PBS could be neglected. **Figure 4.5a** shows the normalized resistance change of SWCNTs before and after antibody immobilization for antibody concentrations of 0.9, 1.8, and 4.5 mg/mL. The normalized resistance change of SWCNTs in PBS was 1.78 while that in antibody solutions varied from 2.04

to 2.12 on average. 78 % of the resistance change was contributed by hydrogen binding, and 30 % was by antibody binding.

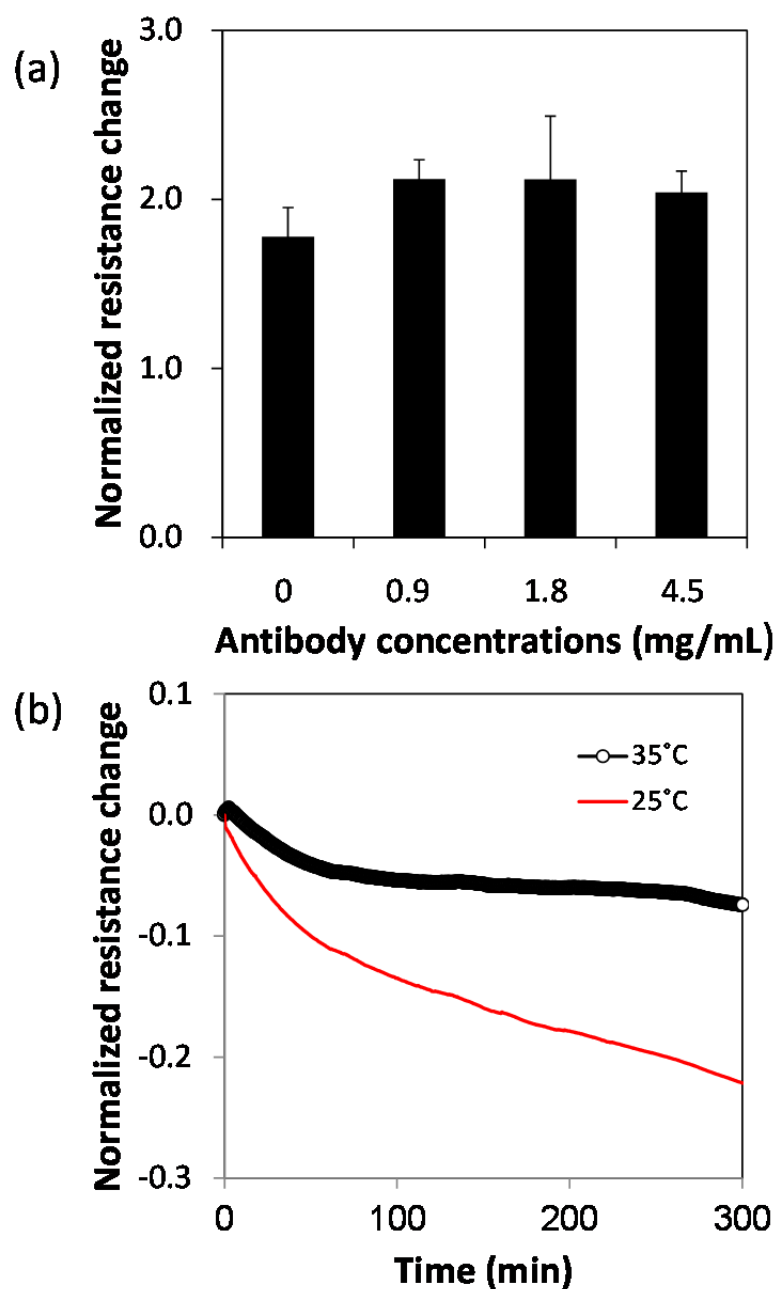


Figure 4.5. (a) Normalized resistance change before and after antibody immobilization on SWCNT sensors (N=4) (a) Normalized resistance change of an SWCNT immunosensor at 25 and 35 °C after antibody immobilization.

To study the resistance change due to antibody binding and proton adsorption, the resistances were measured after antibody immobilization of 24 hours. The resistance rapidly decreased as soon as sensors were taken out of the antibody solution container. When sensors were exposed to air, one batch of the sensors was left in air at 25 °C, and the other batch was heated 35 °C for 300 min. **Figure 4.5b** shows the normalized sensor resistance change at 25 and 35 °C for 300 min. The resistance drop for 25 °C was monotonous while that for 35 °C was larger before 20 min and smaller after 20 min. The larger drop at 35 °C was expected because a larger number of hydrogen were desorbed at a higher temperature. As time went by, the resistance drop at 25 °C became more significant. The resistance decrease also affected the doping level of an SWCNT sensor. For SWCNTs immersed in water, the SWCNTs could be doped with a high concentration of hydrogen and proton, which could dominate the resistance change.

To study the desorption effect of hydrogen on sensor performance, the immunoassay using SWCNT sensors was tested for a sensor after 5, 20, 40, and 120 minutes of incubation at 25 and 35 °C. **Figure 4.6a** shows the change of a normalized resistance for MTB (10^6 CFU/mL) in comparison to the control.

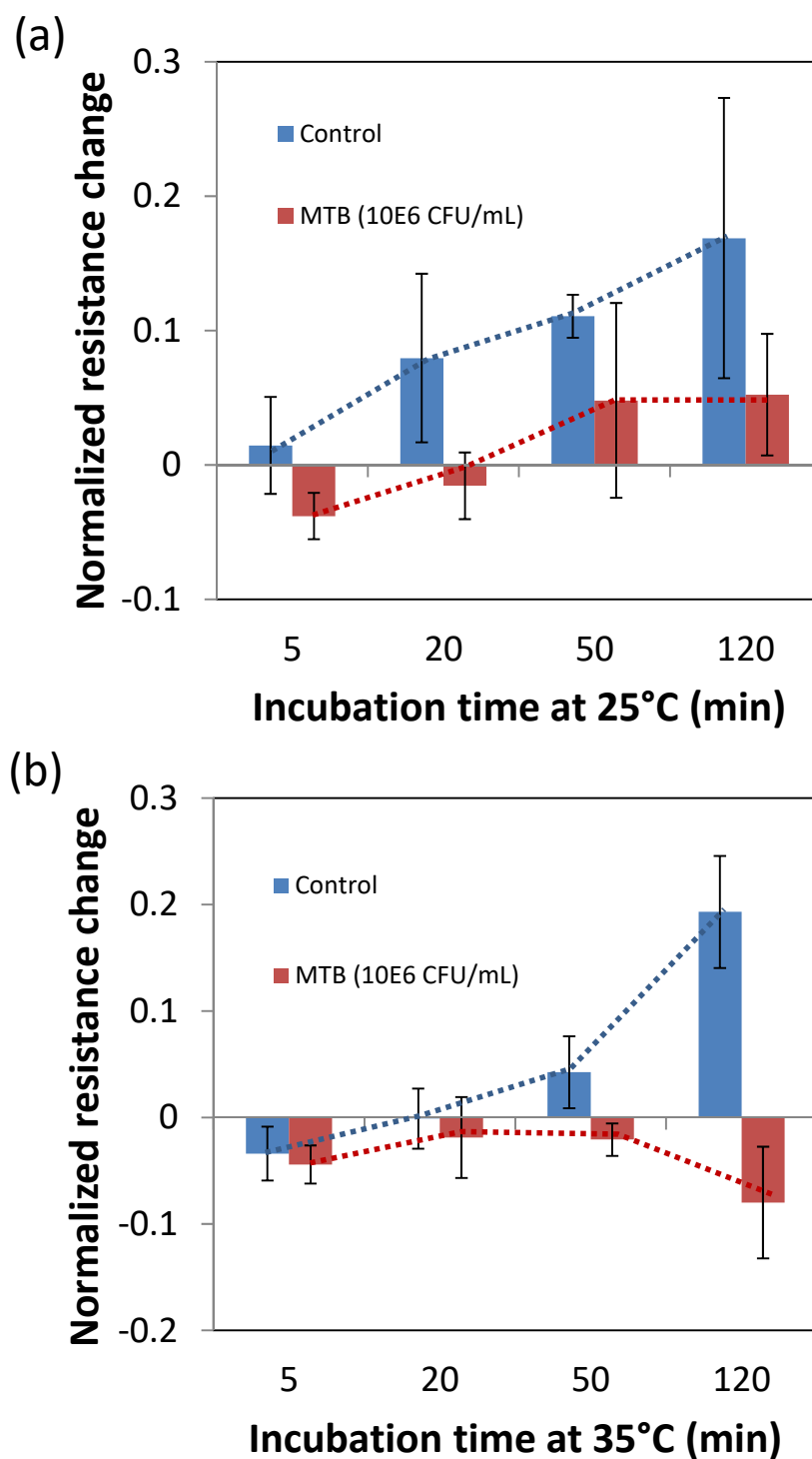


Figure 4.6. (a) The resistance change of an SWCNT immunosensor at 25 and 35 °C after antibody immobilization. (b) Normalized resistance change of a SWCNT sensor for control and

MTB (10^6 CFU/mL) in PBS ($N = 4$). The sensor is tested after 5, 20, 40, and 120 min incubation at 25 °C.

The normalized resistance of the control samples increased for both control and MTB. However, the error bar was too large to differentiate the MTB signal from the control. **Figure 4.6b** shows the normalized resistance change for MTB (10^6 CFU/mL) at 35 °C. The control was negative at 5 min and gradually increased to the positive value. The normalized resistance of the positive MTB samples maintained slightly negative values and dropped to -0.08. When the control was compared with the positive cases, a signal could be detected for the samples of 40 and 120 min incubation at 35 °C. **Figure 4.7** shows the resistance change of an SWCNT sensor after 0.1% PEI coating and antibody coating with 120 min incubation. The resistance of SWCNT sensors increased from 292 to 669 Ω after antibody coating. In a further experiment, the incubation time was set as 120 min for reliable performance of the sensors.

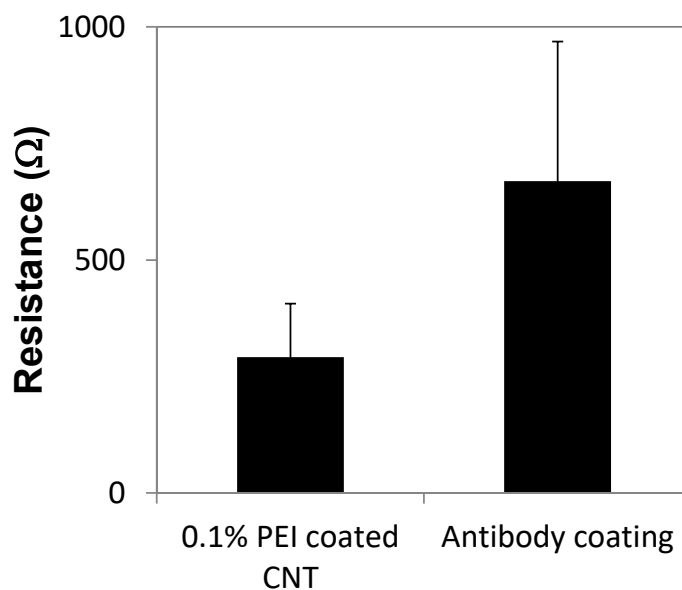


Figure 4.7. Resistances of 0.1% PEI coated SWCNTs and antibody-coated SWCNTs. The resistance is measured after 2 hours at 35 °C.

For a sensitivity test, various concentrations of MTB cells in a PBS buffer were tested, as shown in **Figure 4.8a**. In comparison to the control group, the signal was measured from 10 CFU/mL. In the tests, the normalized resistance change for the control was measured between 0.15 and 0.25 throughout the tests. The average value of the normalized resistance for the control was shifted to 0 for convenience of signal reading. According to the down-shift of the control, the signal was further shifted by the same magnitude. In spite of the high sensitivity, the resistance change was not quantitative in comparison to the MTB concentrations. When the dose-response test was conducted for antigen MPT64 **Figure 4.8b**, the signal was measured from 10 ng/mL.

For a specificity test, the signal of MTB at 100 CFU/mL was clearly differentiated from the control and *S. epi* at 10^3 CFU/mL (**Figure 4.9**). However, *M. Avium* (10^3 CFU/mL) and *M. BCG* (10^3 CFU/mL) showed the signal due to the polyclonal antibodies, which showed the cross-reactivity to *Mycobacterium* strains, including NTM. The cross-reactivity to *Mycobacterium* strains was consistent with that to the ELISA assay.

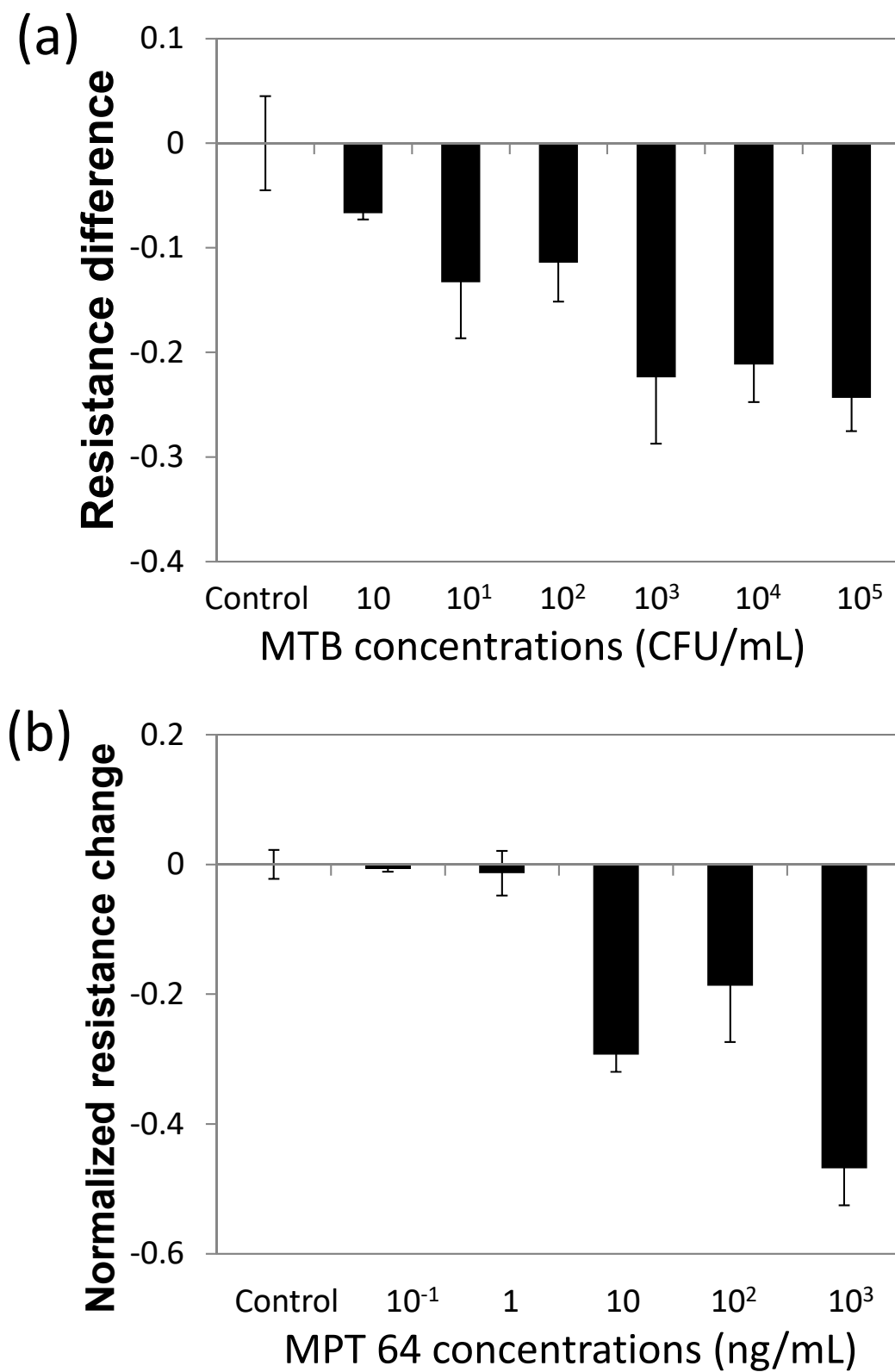


Figure 4.8. (a) Sensitivity test for MTB in PBS. (b) Sensitivity test for MPT64 in PBS.

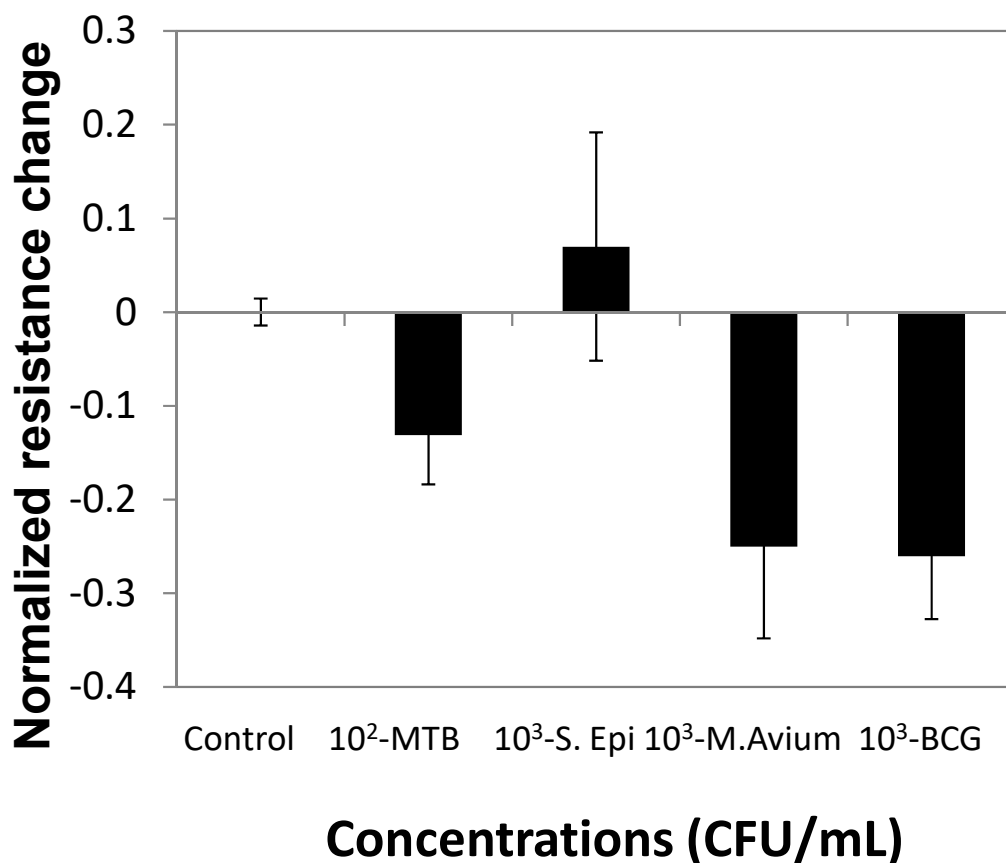


Figure 4.9. Specificity test results for MTB (10^2 CFU/mL), S. Epi (10^3 CFU/mL), M. Avium (10^3 CFU/mL), and M. BCG (10^3 CFU/mL).

To validate if the target cells were captured on a sensor surface, MTB cells (10^6 CFU/mL) stained with SYTO9 green fluorescent dyes (Excitation/Emission: 485/498 nm; LIVE/DEAD BacLight Bacterial Viability Kit, ThermoFisher Scientific) were observed on the sensor surface (**Figure 4.10**). The signal was not clear to detect an individual cell due to the high background fluorescent signal of a PET film and silver surface. However, big colonies could be detected on the SWCNT sensor surface. The qualitative, not quantitative signal could be caused by the

binding nature between bacterial cells and sensor surface. Considering the effective range of electrostatic detection as 10 nm, the nonuniform binding of target cells could cause the qualitative signal. The nonquantitative signal could also be coupled with the large gap size of 500 μm , explaining the saturation of the resistance change in large gap size.

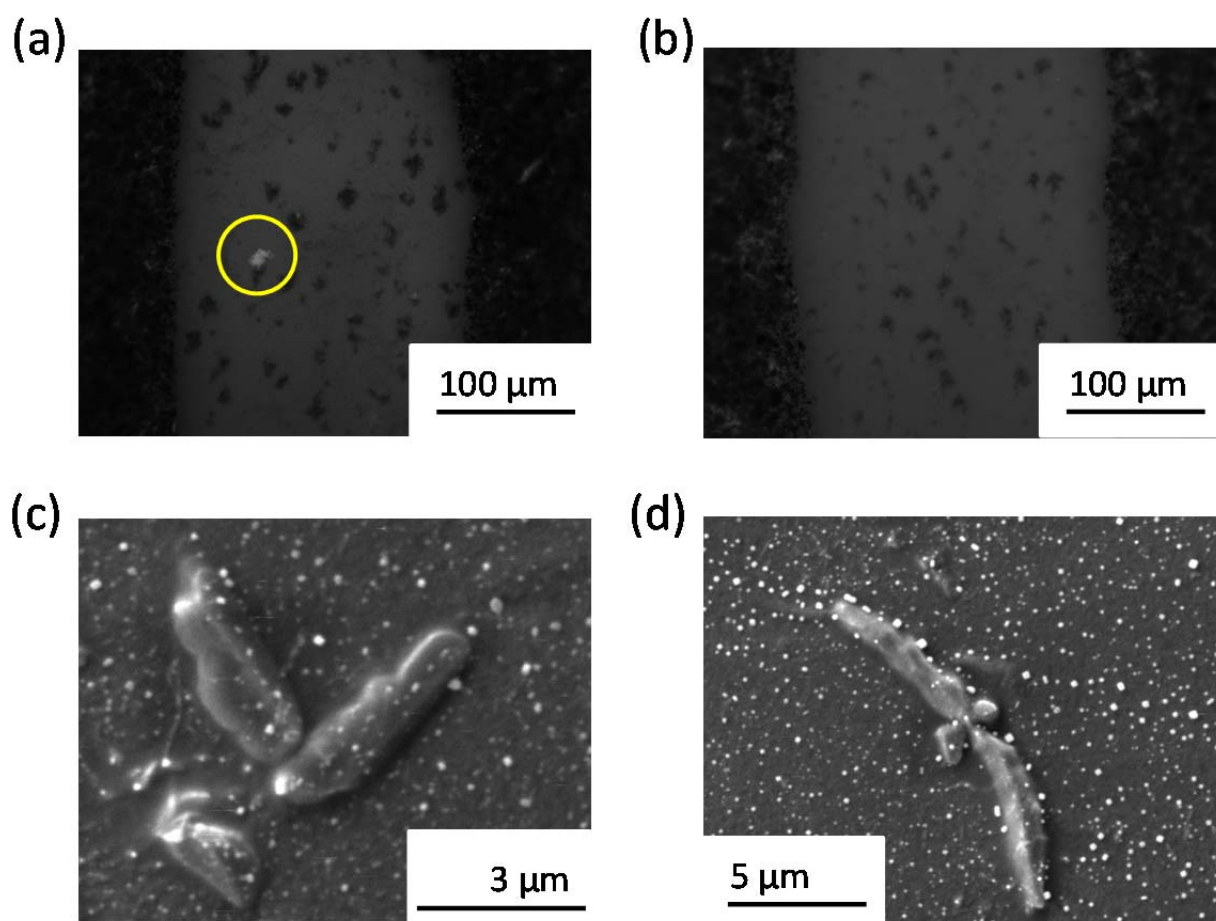


Figure 4.10. (a) Fluorescence microscope images for an MTB colony (10^6 CFU/mL). (b) Control samples. The grey area is the SWCNTs on a PET film. The black area is a silver electrode. (c) and (d) SEM images of MTB cells (10^6 CFU/mL).

To evaluate the LLD for tongue swab samples, MTB at various concentrations from 10 and 10^5 CFU/mL were spiked in tongue swab samples. The detection limit was 10 CFU/mL

(Figure 4.11a). According to the dose-response test, the resistance change was not quantitative but qualitative. For the detection limit test using MTP-64 antigen, the LLD was 100ng/mL, which was also qualitative (Figure 4.11b).

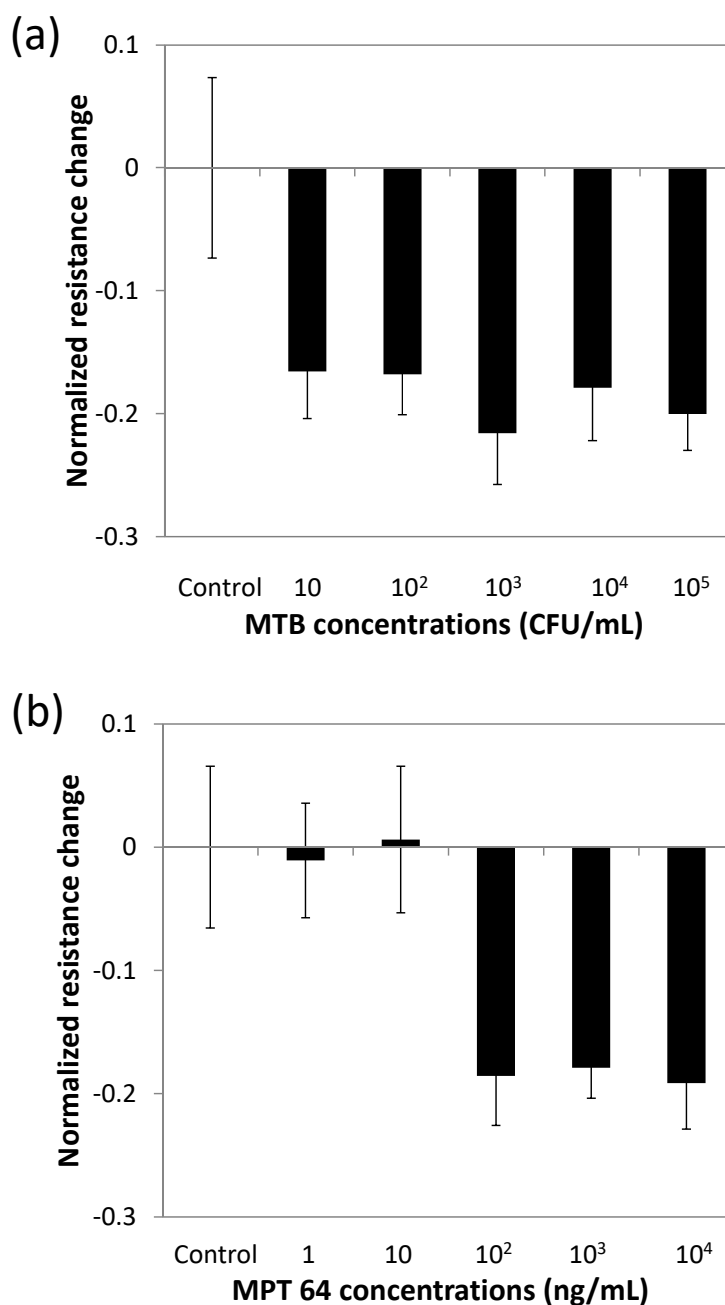


Figure 4.11. Detection limit tests for MTB and MPT 64 (a) MTB spiked in tongue swab samples, (b) MPT64 antigen spiked in tongue swab samples.

Given that tongue swab samples were replete with human cells, bacteria, and other microorganisms, these results also demonstrated the superior specificity of the SWCNT sensor.

The substrate used for the sensor was a PET film. Since the PET film was flexible, the sensor could be attached or bent to fit a testing condition, the resistance change upon bending was tested (**Figure 4.12**).

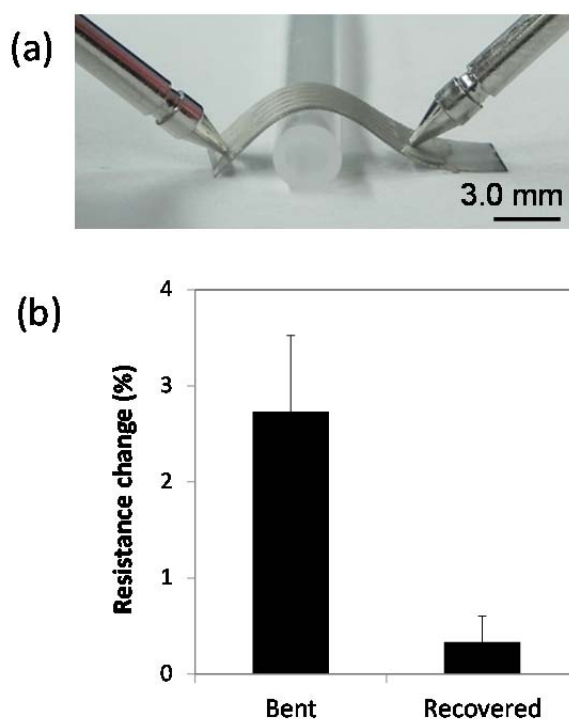


Figure 4.12. (a) Bending test using 3 mm silicone bar (b) Resistance change for the 1st bending and the 1st recovery (N=6).

When the sensor was bent by a radius of 1.5 mm and recovered with the stress release, the resistance change was 0.33 %. In comparison to the change of signal resistance of 10 or more percentage, 0.33 % resistance change could be neglected. However, the resistance change upon

bending was 2.73 %, which could not be neglected. The bending test results show that the sensor could be bent during target binding and operation. However, the measurement should be conducted without external stress. The flexible nature of the sensing platform will benefit the sensor application to platforms requiring a small form factor and low cost.

4.5 CONCLUSIONS

In summary, the resistive immuno-SWCNT sensor was developed to specifically detect *Mycobacterium* cells spiked in tongue swab samples. The detection limit was 10 CFU/mL in tongue swab samples, which was comparable to PCR but without requiring bacteriological culture, centrifugation, or nucleic acid amplification. MPT64 antigen spiked in tongue swab samples was detected at 100 ng/mL. To achieve such high sensitivity and specificity, a challenge of an SWCNT sensor coupled with hydrogen adsorption and desorption was studied to find optimal curing time of 2 hours at 35 °C. Due to the desorption of proton on the SWCNT surface, more stable resistance measurement could be obtained. Unlike other SWCNT-based sensors, the presented sensor was fabricated on a flexible PET film, which offered an extremely low cost and lightweight platform. The small form factor will benefit low-cost diagnosis of TB in military bases and underdeveloped countries. The simple resistive measurement will allow rapid screening by minimally trained personnel within 30 minutes from tongue swab samples. In addition, the minimal power requirement (<1 W) combined with low assay cost is ideal for point-of-care (POC) screening in limited resource settings.

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CLAIMS

Flexible Resistive Single Walled Carbon Nanotube Sensor for Point of Care Screening of diseases

1. A sensor, comprising:
a flexible plastic substrate comprising a template material comprising a carbon nanotube film bonded to the plastic substrate, coated with polymer layers and probe molecules to recognize target analytes, wherein metal electrodes are printed on carbon nanotubes for electrical measurement.
2. The sensor of Claim 1, wherein the plastic substrate is a thin flexible insulating film made of polyethylene terephthalate (PET), polyethylene, cellulose acetate, polypropylene, etc.
3. The sensor of Claim 1, wherein carbon nanotubes are single, double or multi walled carbon nanotubes.
4. The sensor of Claim 3, wherein the polymers are polyethyleneimine (PEI), poly-L-lysine (PLL), or other polymer layers to give charge to carbon nanotubes and work as a linking layer to attach probe molecules.
5. The sensor of Claim 1, wherein carbon nanotubes are heated, vacuumed, or incubated in dry environment to desorb hydrogen molecules that are physisorbed on carbon nanotube surface in aqueous medium.
6. The sensor of Claim 1, wherein the carbon nanotubes are functionalized with probe molecules to capture cells, bacteria, virus, protein, nucleic acid, pesticides, and other molecules.
7. The sensor of Claim 1, wherein the sensor metal electrodes are fabricated by stamping, screen printing, ink jet printing, physical vapor deposition.
8. The sensor of Claim 1, wherein the metal electrodes cover the SWCNTs to eliminate the resistance change at the interface of carbon nanotubes and metal electrodes.

9. The sensor of Claim 1, wherein the SWCNTs cover precious metal electrodes to detect the resistance change at the interface of carbon nanotubes and metal electrodes and carbon nanotubes.

10. The sensor of Claim 1, wherein the resistance or electric current is measured to recognize the binding of the target.

11. The sensor of Claim 1, wherein the magnetic particles bound with target analytes are detected by carbon nanotubes for detection in complex samples.

12. The sensor of Claim 1, wherein the metal electrodes have interdigitated, rectangular, or circular shapes.

13. The sensor of Claim 1, wherein the plastic film substrate is bent for detection in microtubule, sample container, or on a curved surface, such as gloves, clothes, helmets, or skin.

14. The sensor of Claim 1, wherein the resistance change of carbon nanotubes is amplified by the means of charged molecules, electrochemical amplification, and magnetic force.

15. The sensor of Claim 1, wherein the carbon nanotubes are rinsed by deionized water to rinse ions and reagents.

Flexible Resistive Single Walled Carbon Nanotube Sensor for Point-Of-Care Screening of Tuberculosis Using Tongue Swab Samples

Statement of Government Support

This invention was made with government support under Grant No. W81XWH-17-1-0083, awarded by the Department of Defense. The government has certain rights in the invention.

DESCRIPTION

1. Introduction

Tuberculosis, caused by *Mycobacterium tuberculosis* (MTB), is one of the serious infectious diseases worldwide. Although the case number gradually declines, developing countries have shown a significantly higher mortality rate. In Asian and African countries, over 80% of populations show positive cases. For initial TB screening, three sputum samples are collected from a patient in early morning (1). The sample collection procedure is repeated for a few times in order for initial screening. The collected samples are diagnosed with various methods, such as, a Ziehl-Neelsen (ZN) method for microscopic detection, immunoassays for antigen detection(2), polymerase chain reaction (PCR) for DNA or RNA detection. The ZN smear method is labor-intensive, poses a large source of potential errors, has a limited sample throughput, and is not sufficiently sensitive. Enzyme-linked immunosorbent assay (ELISA) is a rapid and relatively easy tool but with low sensitivity. Among the approaches, PCR-based methods have shown clinical sensitivity and specificity greater than 95% but with 2 hours of detection time (3).

In the whole screening time, the significant time for the screening is occupied by the liquefaction process of sputum samples. Since sputum samples are thick and viscous, the samples need to be dissipated potentially with disinfection process for safe screening. The processing time typically requires at least 20 minutes, which has been a major hurdle for point-of-care (POC)

screening of TB. In addition, sputum sample collection has been a challenging problem for kids and babies. Recently, a clinical study has been conducted to discover a new biosample source. Oral swab samples have been found as a convenient noninvasive source for TB diagnosis (4, 5). Using oral swab samples, a POC screening tool can be realized as far as a simple but highly sensitive and specific sensor is offered.

For a highly sensitive and specific screening tool, nanomaterials have been investigated for infectious disease diagnosis. Among the nanomaterials, single walled carbon nanotubes (SWCNTs) are one of the potential candidates to enable a simple resistive transducer to detect the binding of a target analyte with a high sensitivity. In comparison to optical and fluorescent detection, a resistive sensor runs with small power and small form factors. Since optical focus and magnification is not required, a light pocket size device can be developed as a measurement unit. The high sensitivity of a SWCNT biosensor is originated from the small diameter (~ 1 nm) comparable to the size of single biomolecules and the thickness of electrical double layers in physiological buffer(6).

Resistive SWCNT sensors can detect targets by two distinct mechanisms (7-10). One is to change the free carrier density of doped SWCNTs by electrostatic interaction. The other is to change the work function of the metal electrodes-SWCNT interface, thus leading to Schottky barrier modulation. For semiconducting nanotubes, both mechanisms play a role in modulating the resistance. When SWCNTs were deposited on gold electrodes patterned on silicon substrate, viral particles and bacteria could be detected by measuring the resistance change (11, 12). The detection limit of swine influenza virus (H1N1) was 177 TCID (50% Tissue culture Infective Dose)/mL(11). The detection limit for *B. Subtilis* was 100 CFU/mL (12). SWCNTs functionalized with heparin could detect dengue virus as low as 840 TCID/mL (13). The lowest detection limit was 1 PFU/mL

to detect H1N1 (14). In addition, a similar configuration of SWCNT sensor was applied to detect peanut allergen in food extract with the detection limit of 5 ng/mL(15). In mRNA detection, the detection limit was demonstrated in an atto-Molar level, which has a potential to detect nucleic acid without amplification(16). In our previous work, nanotips made of SWCNTs could be used for bacterial detection (17). The crossbar junctions coated with SWCNTs were fabricated to detect target bacteria in food samples (18). The sensor substrate, electrical layers, target samples, detection limit and targets are summarized in Table 1.

Table 1 Resistive single walled carbon nanotubes for detection of bioanalytes

Number	reference	Substrate	Electrical layer	Target sample	Detection limit	Target
1	(14)	Silicon/oxide	On Au	Buffer	1 PFU/mL	H1N1
2	(12)	Silicon/oxide	On Au	Buffer	100 CFU/mL	B. Subtilis
3	(15)	Silicon/oxide	On Au	Food extract	5ng/mL	Ara h 6
4	(11)	Silicon/oxide	On Au	buffer	177 TCID/mL	H1N1 (10, 20 and 50 um gaps)
5	(13)	Silicon/oxide	On Au	buffer	840 TCID/mL	dengue virus
6	(16)	Silicon	On Au	buffer	aM	mRNA
7	(17)	Tungsten wire coated with Au	On Au	buffer	10 ³ CFU/mL	BCG bacteria
8	(18)	Crossing tungsten wires coated with Au	On Au	buffer	10 ² CFU/mL	Escherichia coli

In spite of the great potential as a POC screening sensor, few resistive SWCNT biosensors have been demonstrated for target detection in human samples. One of the main challenges was the screening effect by high ion concentrations in physiological buffer. Positive and negative ions covering SWCNTs rendered both electrostatic and Schottky modulation disabled and

unpredictable. For immunoassay, the challenge was resolved by rinsing with deionized (DI) water. The sensing mechanism of DI-rinsed SWCNTs could be recovered without losing the binding activity of antigen-antibody. In addition to the screening effect, hydrogen binding on SWCNTs in water-based buffer could generate unreliable resistance change due to absorption and desorption of hydrogen. For example, when SWCNTs were bound with antibodies in physiological buffer, hydrogen was bound on SWCNTs to increase the resistance. As soon as the sensor was exposed to air out of buffer, the electrical resistance started to decrease. The resistance change by target bindings could be interfered with that by hydrogen release, which could reduce the sensitivity and reliability.

In this paper, a resistive SWCNT biosensor is fabricated on a flexible film, polyethylene terephthalate (PET) for low cost TB screening. Silver electrodes are stamped as probing electrodes for SWCNTs. The sensing mechanism of SWCNTs coupled with silver electrodes is investigated in conjunction with hydrogen desorption. The sensitivity and specificity are characterized for MTB and surface antigen (MPT64) in PBS. Subsequently, the sensor is characterized for tongue swab samples spiked MTB and MPT64. Simple resistive measurement is conducted before and after immunocomplex formation for detecting targets. The presented biosensor will move one step further to a POC platform for rapid and low-cost TB screening.

2. Experimental method

2.1 Sensor fabrication

The sensor was fabricated on a polyethylene terephthalate (PET) film (Fig. 1a). Target cells and antigen are detected by using a SWCNT sensor functionalized with Polyethyleneimine (PEI) and antibodies (Fig. 1b). Silver electrodes are patterned for resistive detection. When

targets are bound on the sensor surface, the resistance decreases due to the electrostatic interaction.

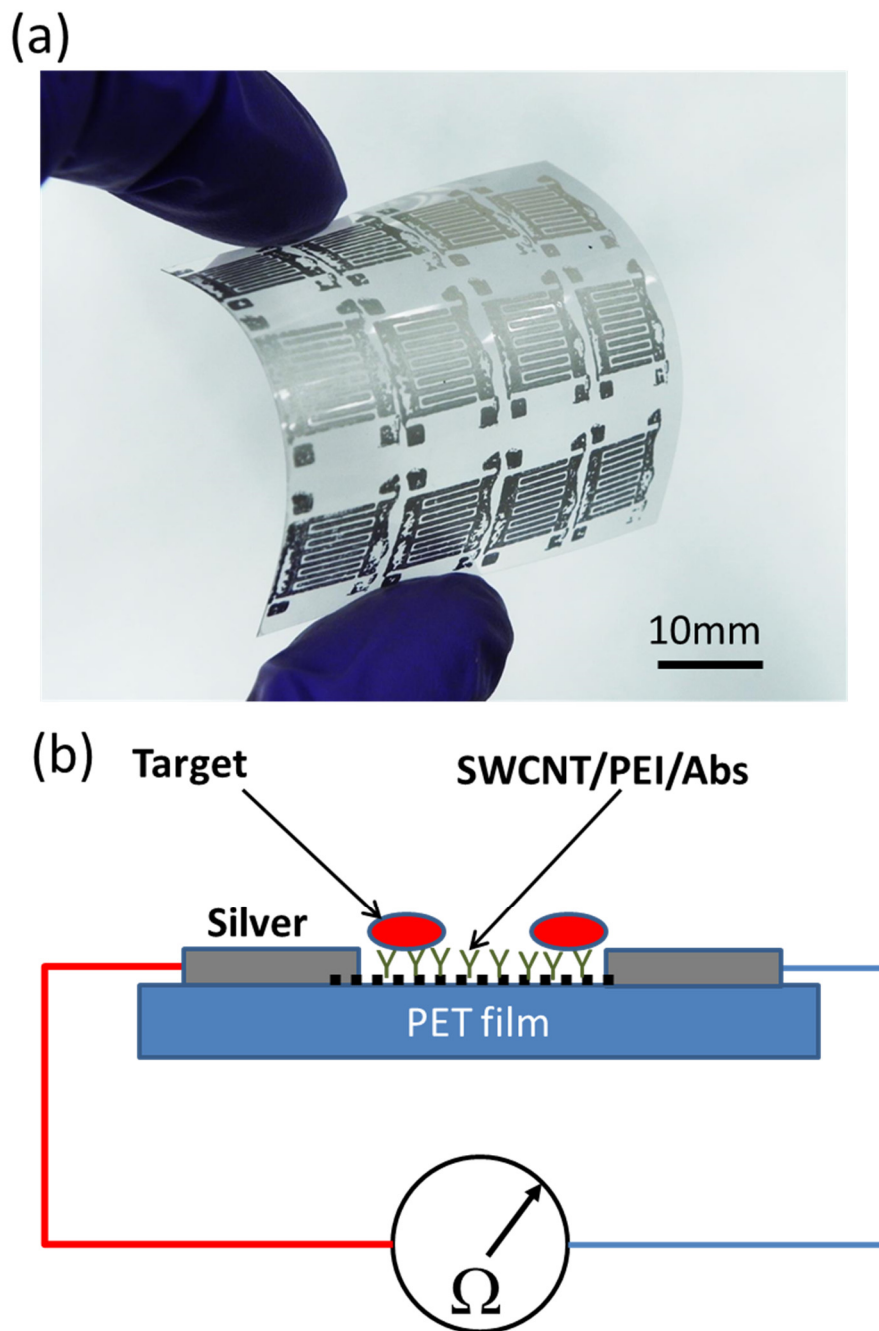


Fig. 1 (a) SWCNT-based sensor on a flexible PET film. (b) Cross section of a resistive SWCNT immuno sensor.

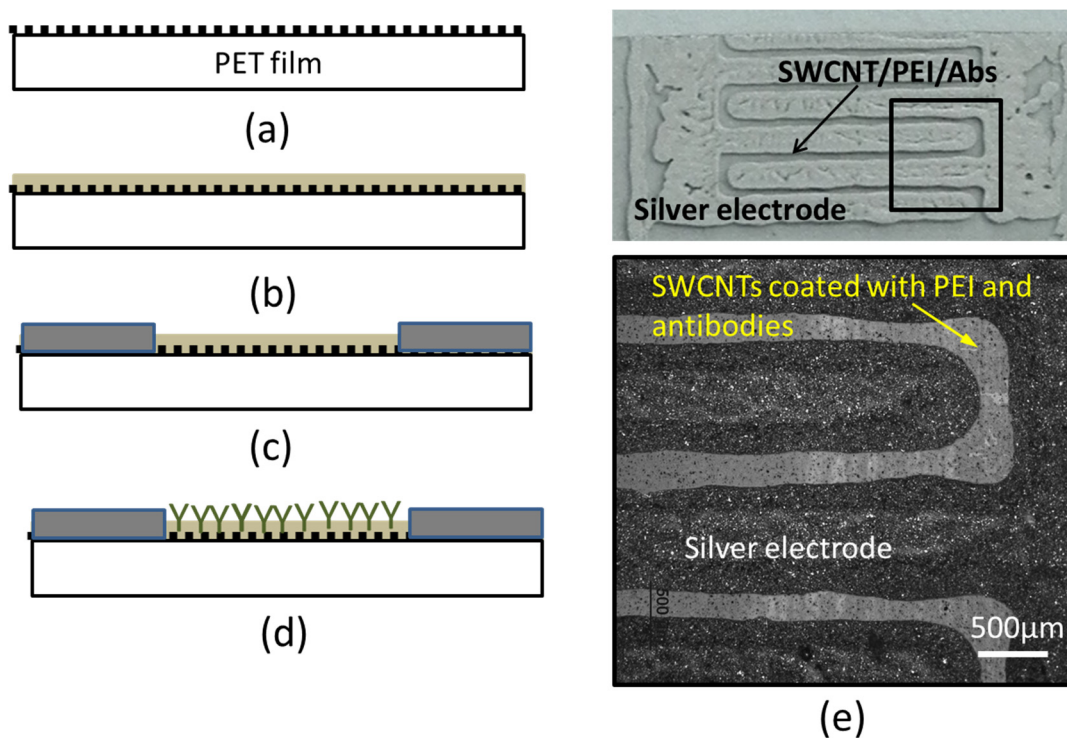


Fig. 2 Fabrication process of a SWCNT-based immunosensor (a) Spin coating of SWCNTs on a PET film (b) Spin coating of PEI (c) Stamping of silver electrodes. (d) Antibody immobilization (e) Photo and optical microscope images of a SWCNT immunosensor.

For fabrication (Fig. 2a~2d), SWCNTs were dispersed in SDS at a concentration of 1mg/mL using a sonicator at room temperature for 8 hours. The SWCNTs were spin-coated on a PET film at 6000 rpm for 1 minute. PEI (0.1%) was coated on the SWCNT surface. Silver ink was stamped on the PEI coated SWCNT surface. For silver electrode patterning, a Delrin® mold was machined by using an end mill. The stamp was made of polydimethylsiloxane (PDMS) cured in a mold at room temperature for 3 days. The PDMS stamp coated with silver ink was stamped

on a PEI-coated SWCNT sensor. The sensor was cured on a hot plate for 1 hour at 100°C. A polyclonal IgY antibody (18 mg/mL) raised against MPT64 protein was physisorbed on the SWCNT surface for 24 hours in refrigerator (4°C). Subsequently, one sensor was cut by scissors by half in order to make 2 sensors. With the cutting, total 24 sensors could be fabricated on a 40x40 mm² PET film. Fig. 2e shows a photo showing a sensor composed of two interdigitated electrodes. The gap is filled with functionalized SWCNTs. The magnified image shows the gap size of 200~300 μm.

Unlike other SWCNT immunosensors, the SWCNTs in a sensor were coated with silver electrodes, which minimized the exposure of the interfacial area between SWCNTs and silver electrodes. The sealing of the interfacial area could offer a uniform contact resistance and isolate Schottky effect in the sensing mechanism. The electrostatic gating effect was only the mechanism to detect the target analytes. In the configuration, the oxidation of silver electrode surface should not interfere the resistive change for target detection.

2.2 Antibody preparation

A polyclonal IgY antibody (pAb) was raised against purified MPT64 protein in Aves Labs (Davis, CA, USA). Complete Freund's adjuvant was used; thus antibodies were reactive to MTB as well as MPT64. The antibodies were raised in two hens and evaluated by ELISA to determine binding to protein, and filter plate EIA to determine reactivity to target cells.

To evaluate antibodies against *Mycobacterium*, cultures of BCG and MTB H37Ra cells were diluted to 1×10^7 cells/mL in PBS. Cell solutions were then added to a 96-well filter bottom plate (Millipore 0.45 μm, #MAHVN4510), 100 μL of a 1×10^7 cells/mL solution of MTB or BCG. The cells were trapped by the 0.45-micron filter, and buffer/media was washed. Cells were washed

with vacuum filtration, 3x200ul DPBS. 28 µg/mL IgY antibody (100ul) was added to each well and incubated for 30 min @37°C. Control (pre-immune IgY) antibodies were tested at the same concentration. The IgY solution was washed with 4x200ul DPBS. A 100µl solution of a 2° antibody (1:1000 dilution Rab anti-IgY-HRP Conjugate, Thermo Scientific #31401) was added and incubated for 30 min @37°C, followed by DPBS washing (3x200 µL). Finally, 100µl ABTS substrate (Thermo Scientific #37615) was added followed by a 10-minute incubation at room temperature. The solution was then filtered through the filter plate and into a clear 96-well plate and read at A₄₀₅ in a microplate reader. Fig. 3a shows the positive results of the antibodies for both BCG and MTB H37Ra, According to the results, the polyclonal antibodies were specific to Mycobacterium strains potentially including non-tuberculosis Mycobacterium (NTM) species. The specificity test will be conducted using a SWCNT immunosensor.

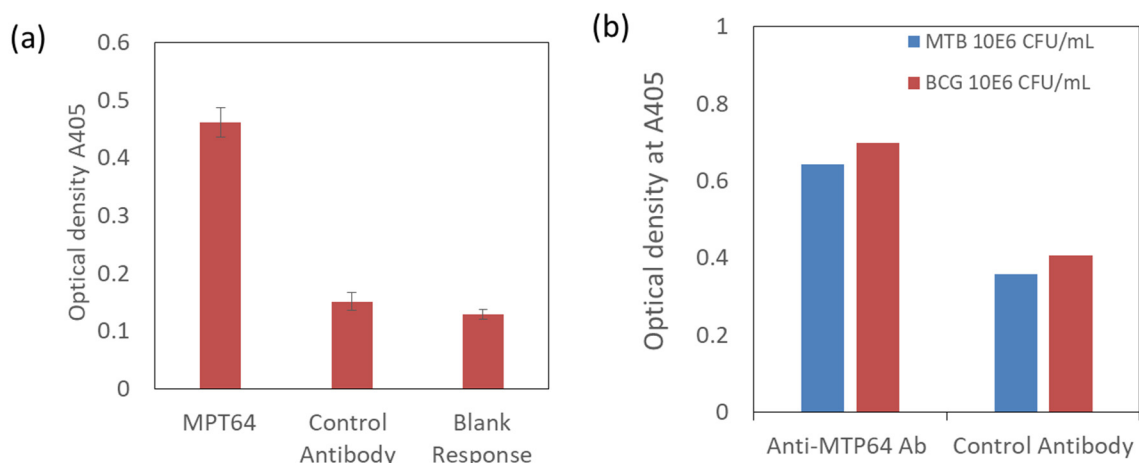


Fig. 3 (a) Optical density showing the binding of MPT64 antibodies to MTB (10^6 CFU/mL) and BCG (10^6 CFU/mL) at 28µg/mL. (b) Optical density showing the binding of MPT64 antibodies to MPT 64 in comparison to control

To assay the MPT 64 protein, 100 μ L of a 100 μ g/mL solution of MPT-64 in DPBS was added to an ELISA protein binding 96-well plate (Immulon 2HB, Thermo Scientific #3455). The mixture was incubated overnight at room temperature, followed by 1H at 37C, and then washed with 3x200 μ L DPBS. To block the remaining sites in the well, a 200 μ L BSA solution in DPBS at 1mg/ml was added and incubated for 1 hour at 37°C followed by washing with 3x200 μ L DPBS. A 100 μ L solution of IgY raised to MPT-64 was added to each well at a concentration of 28 μ g/ml in DPBS. Control (pre-immune IgY) antibodies were tested at the same concentration. A 100 μ L solution of a 1:1000 Dilution of 2° (Rab anti-IgY-HRP Conjugate, Thermo Scientific #31401) was then added and incubated for 30 min @37C, followed by DPBS washing (3x200 μ L). Finally, 100 μ L of ABTS substrate (Thermo Scientific #37615) was added and measured at A₄₀₅ after a 10-minute incubation at room temperature. Fig. 3b shows the positive results from the antibodies to MPT-64 compared to pre-immune antibodies.

2.3 Sensor characterization

After antibody immobilization, the resistance of SWCNT sensors increased due to antibody binding and hydrogen binding on SWCNTs. To study the contribution of resistance increase in an antibody immobilization step, the antibody concentration varied from 0, 1.4, 2.8 and 7.0 μ g/mL in PBS buffer. After 24 hours of incubation of SWCNT sensors in each solution, the normalized resistance change was measured before and after antibody immobilization.

As soon as the sensor was exposed to air, the sensor resistance started to decrease due to desorption of hydrogen. The desorption process was critical to obtain a reproducible resistance measurement after binding targets. To characterize the resistance change due to the hydrogen desorption, the resistance change was measured for 5 hours. One sensor was incubated at 25 °C,

and the other sensor was incubated at 35°C on a hot plate. The relative humidity was 25~30% in the tests. Since antibodies were immobilized on the sensor surface, the sensor temperature was not tested over 35 °C in order to avoid potential damage or degradation of antibodies.

To characterize how the sensor response was changed due to hydrogen desorption, the sensor response to targets (MTB at 10^6 CFU/mL in PBS) binding was tested after 5, 20, 40 and 120 minutes of incubation at both 25 and 35°C. The sensor response for MTB at 10^6 CFU/mL in PBS was compared for each condition in comparison to the control (PBS). The incubation time of 5, 20, 40 and 120 minutes was determined in consideration of the slope change of the resistance at 35°C.

2.3 Sensitivity and specificity tests

For sensitivity and specificity tests, both MTB and MPT64 were suspended in 1x PBS buffer. For MTB, various concentrations of MTB cells were suspended in PBS from 10^1 ~ 10^5 CFU/mL. MPT64 was also suspended in PBS from 0.1 ng/mL to 1µg/mL with 10 fold dilutions. 1mL of each solution was supplied in each plastic cup where a sensor was dipped for immunocomplex formation. After 10 min of incubation, the sensor was rinsed with DI water. After gentle blow dry with nitrogen, the resistance was measured. The resistance values before and after immunocomplex formation were R_0 and R_t , respectively. The normalized resistance change $[(R_t - R_0)/R_0]$ was computed to compare the normalized resistance change from control.

For specificity test, the response for MTB (10^2 CFU/mL) was compared with *Staphylococcus epidermidis* (S. Epi at 10^3 CFU/mL), *Mycobacterium Avium* (M. Avium at 10^3 CFU/mL), and *Mycobacterium Bacillus Calmette–Guérin* (M. BCG at 10^3 CFU/mL). Each bacterium species was suspended in 1mL-PBS.

2.4 Test using tongue swab samples

As described earlier, a tongue swab sample was one of new types of oral swab samples that contained MTB markers. To evaluate LLD for MTB and MPT 64 in tongue swab samples, swab samples were prepared by scraping tongue surface from deidentified volunteers as illustrated in Fig. 4. After complete dry of swabs in air, the swab samples were immersed in 1mL PBS contained in an individual microtube for 20 minutes with gentle stirring. Subsequently, 500 μ L of target analytes (MTB or MPT 64) in PBS was mixed with 500 μ L of the eluted swab solution. The 1mL solution was used to test LLD. The concentrations of MTB were $10\sim 10^5$ CFU/mL with 10 fold dilutions. The concentrations of MPT64 ranged from 1ng/mL to 10 μ g/mL with 10 fold dilutions. A sensor was incubated in 1mL sample solutions for 10 minutes. After rinsing with DI water, the resistances were measured. Before and after the incubation, the resistances were measured to compute a normalized resistance.

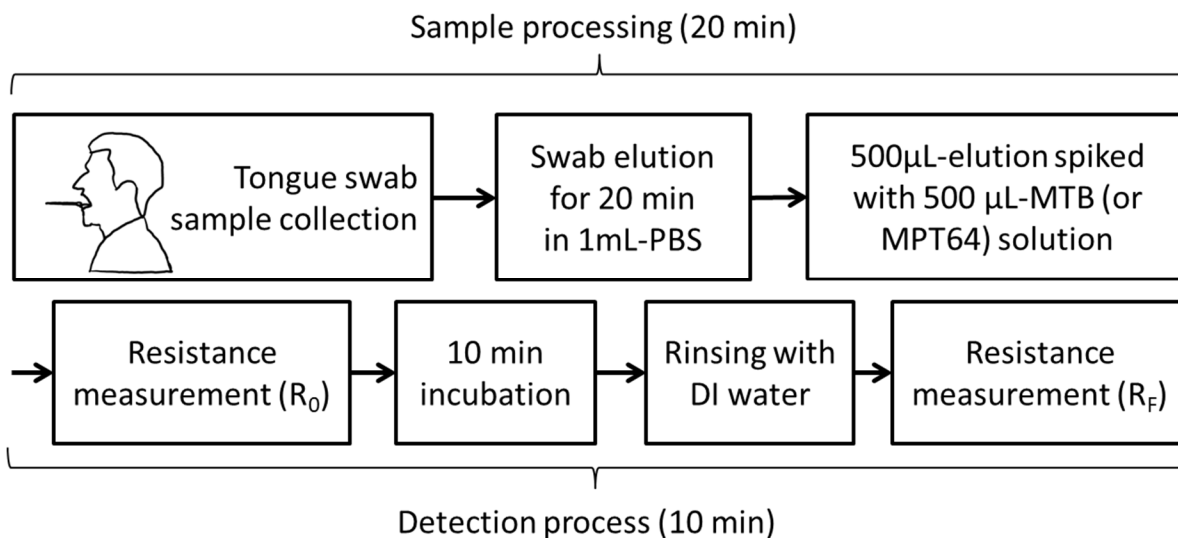


Fig. 4 Preparation protocol of tongue swab samples and resistive detection procedure.

3. Experimental Results

In the antibody immobilization step of 24 hours, the resistance of SWCNT sensors was changed by the bindings of both hydrogen and antibodies. Since the most ions in PBS were washed in the rinsing step after antibody immobilization binding, the effect of ions in PBS could be neglected. Fig. 5a shows the normalized resistance change of SWCNTs before and after antibody immobilization for antibody concentrations of 1.4, 2.8, and 7.0 $\mu\text{g/mL}$. The normalized resistance change of SWCNTs in PBS was 1.78 while that in antibody solutions varied from 2.04 to 2.12 in average. 78% of the resistance change was contributed by hydrogen binding, and 30% was by antibody binding.

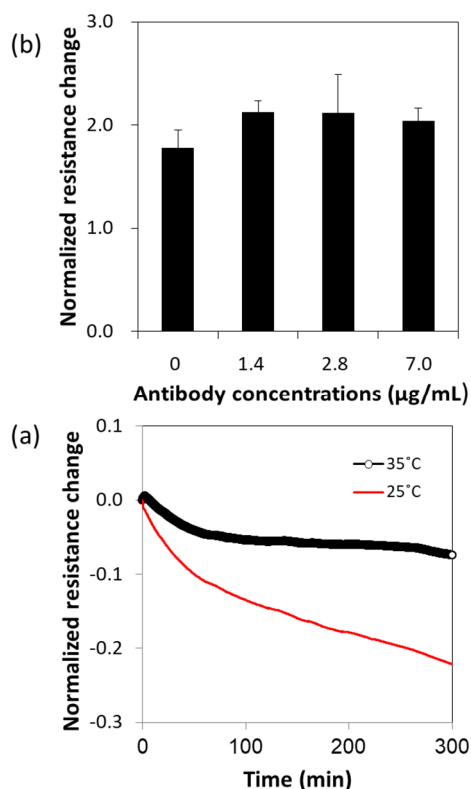


Fig. 5 (a) Normalized resistance change before and after antibody immobilization on SWCNT sensors (N=4) (a) Normalized resistance change of a SWCNT immunosensor at 25 and 35 °C after antibody immobilization.

To study the resistance change due to antibody binding and hydrogen adsorption after immobilization, the resistances were measured after antibody immobilization of 24 hours. The resistance rapidly decreased as soon as sensors were taken out of the antibody solution container. When sensors were exposed to air, one batch of the sensors was left in air at 25 °C, and the other batch was heated 35 °C for 300 min. Fig. 5b shows the normalized sensor resistance change at 25 and 35 °C for 300 min. The resistance drop for 25 °C was monotonous while that for 35 °C was larger before 20 min and smaller after 20 min. The larger drop at 35 °C was expected because a larger number of hydrogen molecules were desorbed at the higher temperature. As time went by, the resistance drop at 25 °C became more significant. The resistance decrease also affected the doping level of a SWCNT sensor. For SWCNTs immersed in water, the SWCNTs could be doped with high concentration of hydrogen and proton, which could dominate the resistance change.

To study the desorption effect of hydrogen on sensor performance, the immunoassay using SWCNT sensors was tested for a sensor after 5, 20, 40 and 120 minutes of incubation at 25 and 35 °C. Fig. 6(a) shows the change of a normalized resistance for MTB (10^6 CFU/mL) in comparison to the control. The normalized resistance of the control samples increased for both control and MTB. However, the error bar was too large to clear differentiate the MTB signal from the control. Fig. 6(b) shows the normalized resistance change for MTB (10^6 CFU/mL) at 35 °C. The control was negative at 5 min and gradually increased to the positive value. The normalized resistance of the positive MTB samples maintained slightly negative values and dropped to -0.08. When the control was compared with the positive cases, a signal could be detected for the samples of 40 and 120 min incubation at 35 °C. Fig. 6(c) shows the resistance change of a SWCNT sensor after 0.1% PEI coating and antibody coating with 120 min incubation. The resistance of SWCNT

sensors increased from 292 to 669 Ω after antibody coating. In further experiment, the incubation time was set as 120 min for reliable performance of the sensors.

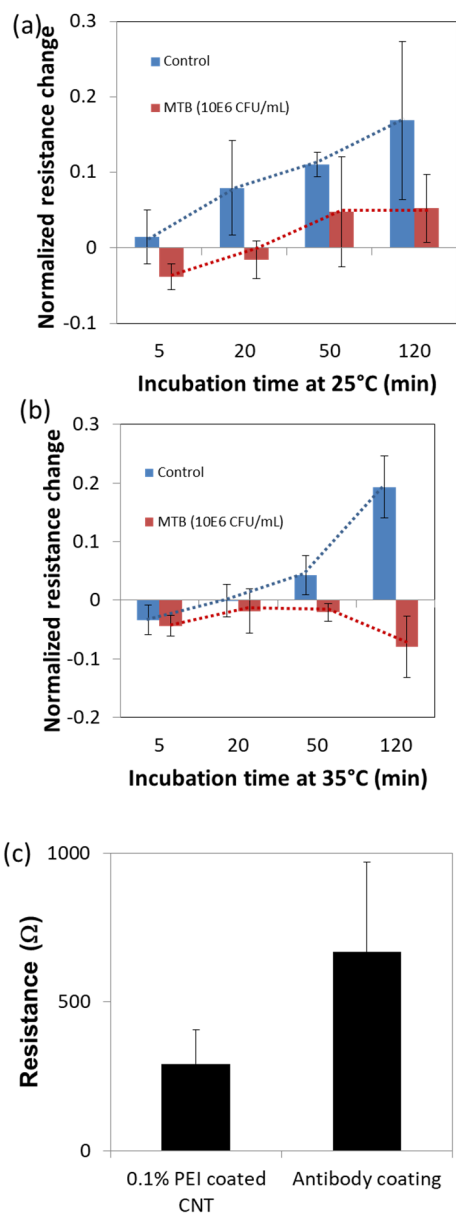


Fig. 6 (a) Normalized resistance change of a SWCNT sensor for control and MTB (10^6 CFU/mL) in PBS (N=4). The sensor is tested after 5, 20, 40, and 120 min incubation at 25°C. (b) Normalized resistance change of a SWCNT sensor for control and MTB (10^6 CFU/mL) in PBS (N=4). The sensor is tested after 5, 20, 40, and 120 min incubation at 35°C. (c) Resistances of 0.1% PEI coated SWCNTs and antibody coated SWCNTs. The resistance is measured after 2 hours at 35°C.

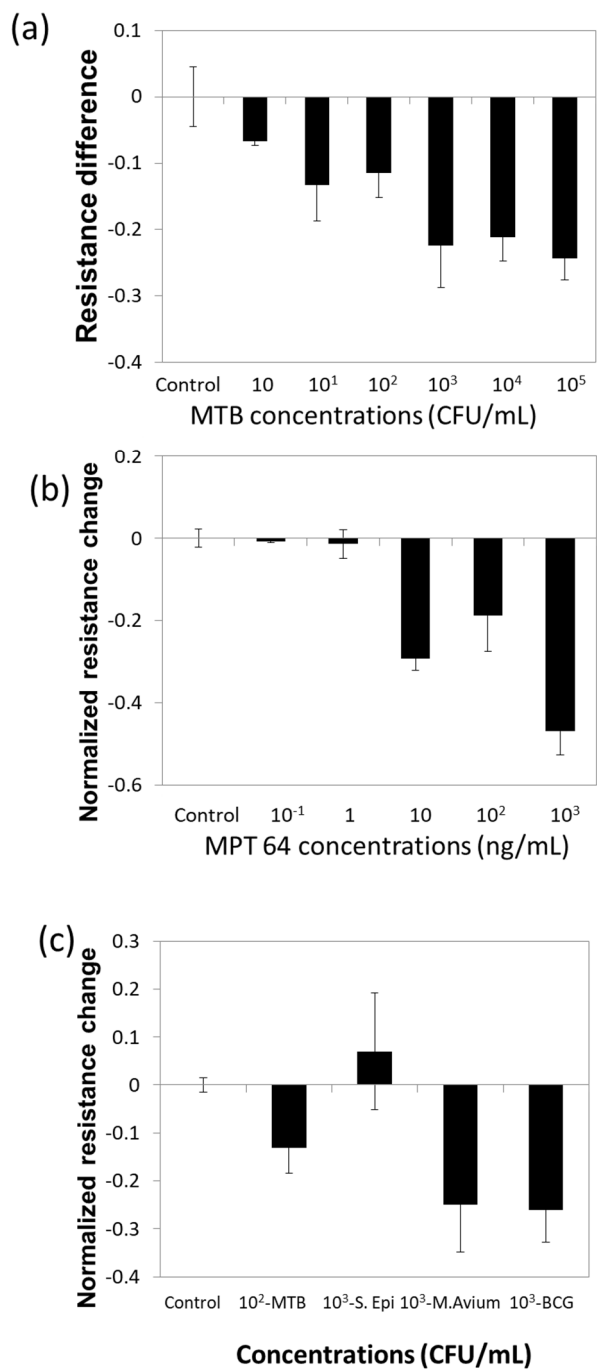


Fig. 7 (a) Sensitivity test for MTB in PBS. (b) Sensitivity test for MPT64 in PBS. (c) Specificity test results for MTB (10² CFU/mL), S. Epi (10³ CFU/mL), M. Avium (10³ CFU/mL), and M. BCG (10³ CFU/mL).

For a sensitivity test, various concentrations of MTB cells in a PBS buffer were tested as shown in Fig. 7a. In comparison to the control group, the signal was measured from 10 CFU/mL. In the tests, the normalized resistance change for the control was measured between 0.15 and 0.25 throughout the tests. The average value of the normalized resistance for the control was shifted to 0 for convenience of signal reading. According to the down-shift of the control, the signal was further shifted by the same magnitude. In spite of the high sensitivity, the resistance change was not quantitative in comparison to the MTB concentrations. When the dose response test was conducted for antigen MPT64 (Fig. 7b), the signal was measured from 10 ng/mL.

For a specificity test, the signal of MTB at 100 CFU/mL was clearly differentiated from the control and *S. epi* at 10^3 CFU/mL (Fig. 7c). However, *M. Avium* (10^3 CFU/mL) and *M. BCG* (10^3 CFU/mL) showed the signal due to the polyclonal antibodies, which showed the cross-reactivity to *Mycobacterium* strains including NTM. The cross reactivity to *Mycobacterium* strains was consistent with that to the ELISA assay.

To validate if the target cells were captured on a sensor surface, MTB cells (10^6 CFU/mL) stained with SYTO9 green fluorescent dyes (Excitation/Emission: 485/498 nm; LIVE/DEAD BacLight Bacterial Viability Kit, ThermoFisher Scientific) were observed on the sensor surface (Fig. 8a). The signal was not clear to detect an individual cell due to the high background fluorescent signal of a PET film and silver surface. However, big colonies could be detected on SWCNT sensor surface. The qualitative not quantitative signal could be caused from the binding nature between bacterial cells and sensor surface. Considering the effective range of electrostatic detection as 10 nm, the nonuniform binding of target cells could cause the qualitative signal. The nonquantitative signal could also be coupled with the large gap size of 500 μ m, explaining the

saturation of the resistance change in a large gap size. Individual colonies were detected by scanning electron microscope (SEM) (Fig. 8b and 8c).

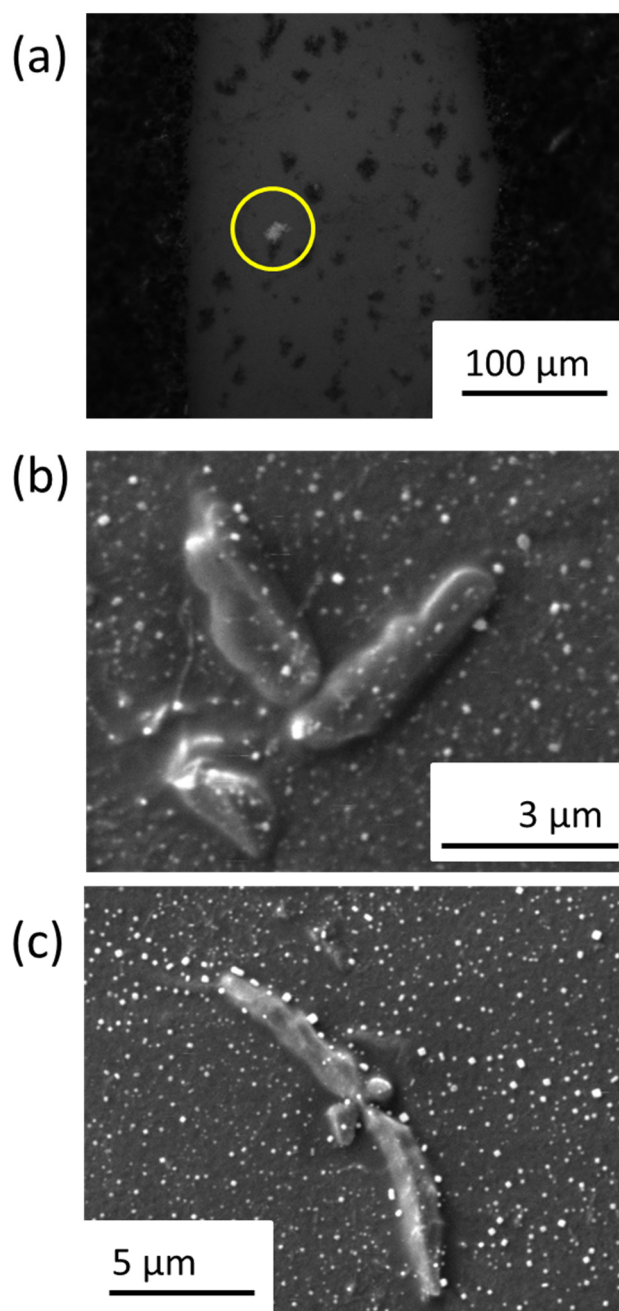


Fig. 8 (a) Fluorescence microscope images for a MTB colony (10^6 CFU/mL). The grey area is the SWCNTs on a PET film. The black area is silver electrode. (b) and (c) SEM images of MTB cells (10^6 CFU/mL).

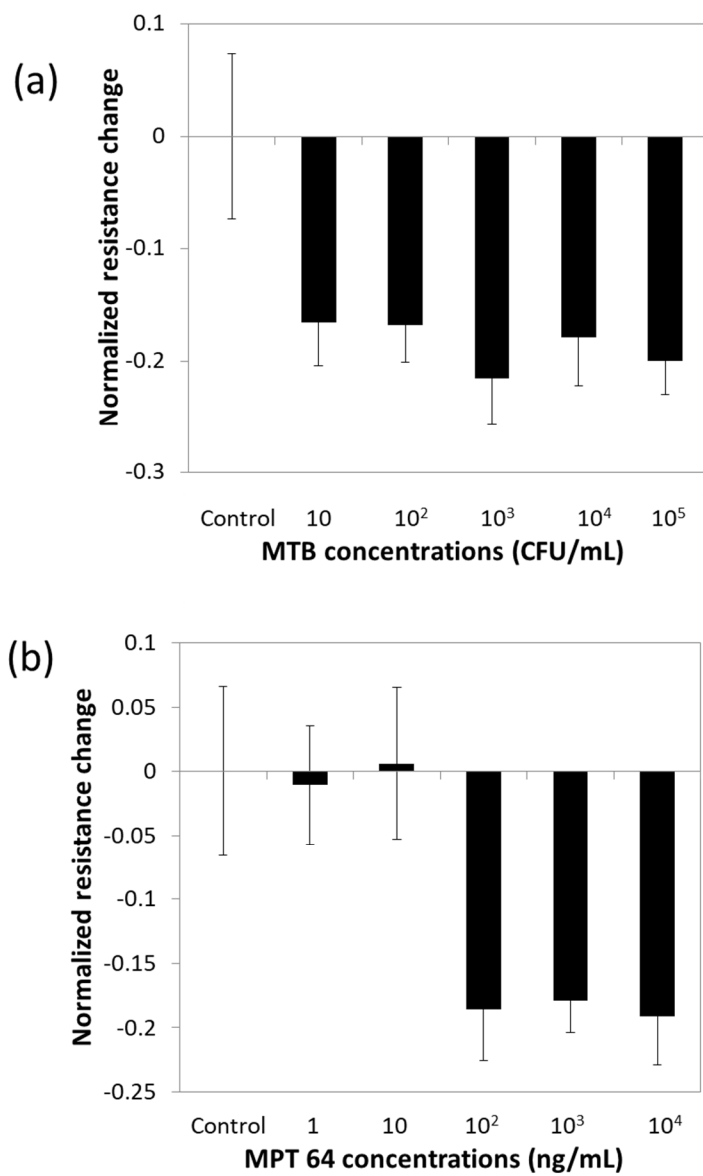


Fig. 9 Detection limit tests for MTB and MPT 64 (a) MTB spiked in tongue swab samples (b) MPT64 antigen spiked in tongue swab samples.

To evaluate the LLD for tongue swab samples, MTB at various concentrations from 10 and 10^5 CFU/mL were spiked in tongue swab samples. The detection limit was 10 CFU/mL (Fig. 9a). According to the dose response test, the resistance change was not quantitative but qualitative. For the detection limit test using MTP-64 antigen, the LLD was 100ng/mL, which was also qualitative (Fig. 9b). Given that tongue swab samples were replete with human cells, bacteria, and other microorganisms, these results also demonstrated the superior specificity of the SWCNT sensor.

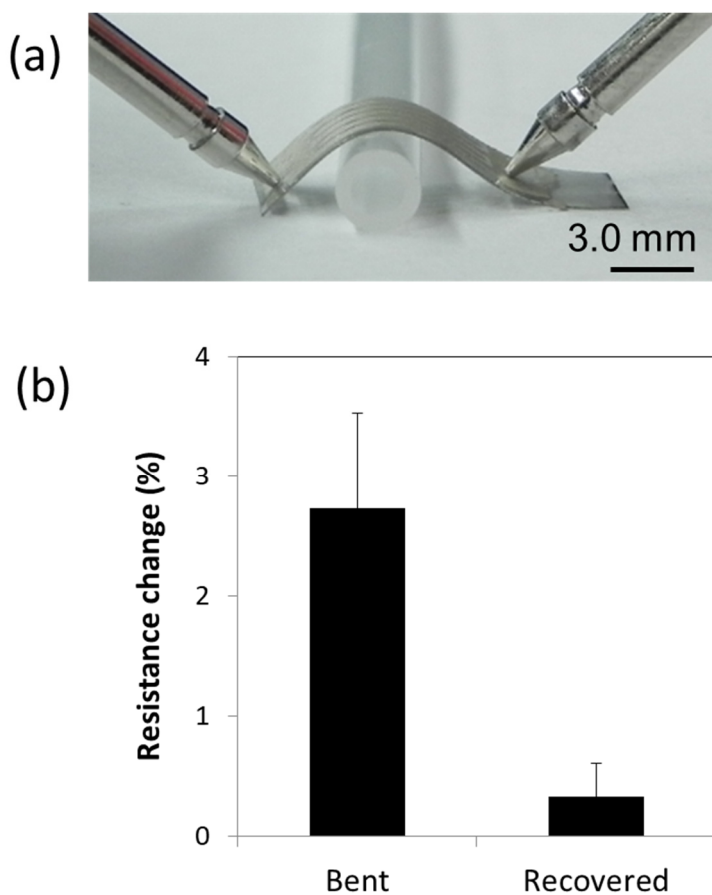


Fig. 10 (a) Bending test using 3 mm silicone bar (b) Resistance change for the 1st bending and the 1st recovery (N=6).

The substrate used for the sensor was a PET film. Since the PET film was flexible, the sensor could be attached or bent to fit a testing condition, the resistance change upon bending was tested. When the sensor was bent by a radius of 1.5 mm and recovered with the stress release, the resistance change was 0.33 %. In comparison to the change of signal resistance of 10 or more percentage, 0.33 % resistance change could be neglected. However, the resistance change upon bending was 2.73%, which could not be neglected. The bending test results show that the sensor could be bent during target binding and operation. However, the measurement should be conducted without external stress. The flexible nature of the sensing platform will benefit the sensor application to platforms requiring a small form factor and low cost.

Conclusion

In summary, the resistive immuno-SWCNT sensor was developed to specifically detect Mycobacterium cells spiked in tongue swab samples. The detection limit was 10 CFU/mL in tongue swab samples, which was comparable to PCR but without requiring bacteriological culture, centrifugation, or nucleic acid amplification. MPT64 antigen spiked in tongue swab samples was detected at 100 ng/mL. To achieve such a high sensitivity and specificity, a challenge of a SWCNT sensor coupled with hydrogen adsorption and desorption was studied to find optimal curing time of 2 hours at 35°C. Due to the desorption of proton on SWCNT surface, more stable resistance measurement could be obtained. Unlike other SWCNT-based sensors, the presented sensor was fabricated on a flexible PET film, which offered an extremely low cost and light weight platform. The small form factor will benefit low cost diagnosis of TB in underdeveloped countries. The simple resistive measurement will allow rapid screening by minimally trained personnel within 30

minutes from tongue swab samples. In addition, the minimal power requirement (<1 W) combined with low assay cost is ideal for point-of-care (POC) screening in limited resource settings.

Resistive SWCNT Sensor Combined with Magnetic Enrichment for Point-Of-Care Screening of Tuberculosis

1.1. Sensor fabrication

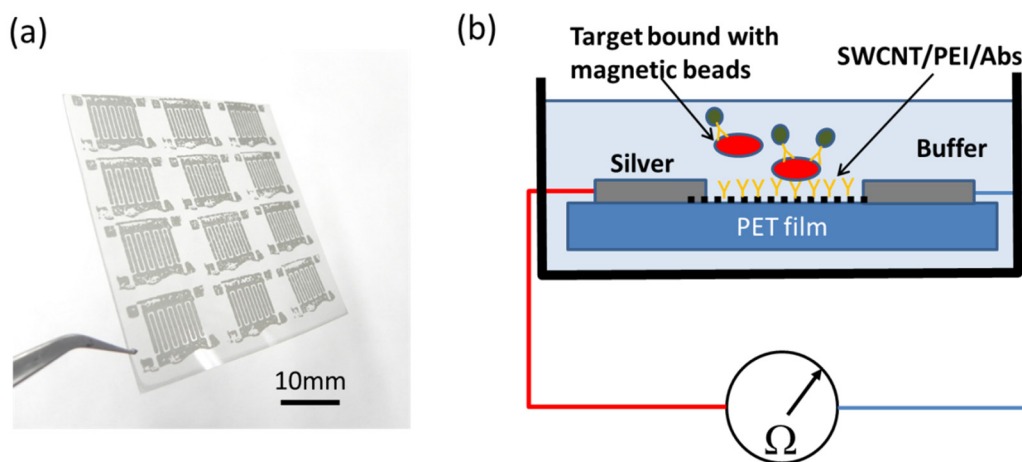


Fig. 11 (a) SWCNT-based sensor on a flexible PET film. (b) Working principle of a resistive SWCNT immuno sensor.

The sensor was fabricated on a polyethylene terephthalate (PET) film (Fig. 11). For fabrication (Fig. 12), SWCNTs were dispersed in SDS at a concentration of 1mg/ml using a sonicator at room temperature for 8 hours. The SWCNTs were spin-coated on a PET film at 6000 rpm for 1 minute. Polyethyleneimine (1% PEI) was coated on the SWCNT surface. Silver ink was stamped on the PEI coated SWCNT surface. For silver electrode patterning, a Delrin® mold was machined by using an end mill. The stamp was made of polydimethylsiloxane (PDMS) cured in a mold at room temperature for 3 days. The PDMS stamp coated with silver ink was stamped on the PEI coated SWCNT sensor. The sensor was cured on a hot plate for 1 hour at 100°C. A polyclonal IgY antibody (1mg/mL) raised against MPT64 protein was physisorbed on the SWCNT surface for 24 hours in refrigerator (4°C). The sensor array was cured on a hot plate (35°C) for 2 hours.

Subsequently, one sensor was cut by scissors in half in order to make 2 sensors. Since 12 sensors were fabricated on a PET film, total 24 sensors could be fabricated on a 40x40 mm² PET film.

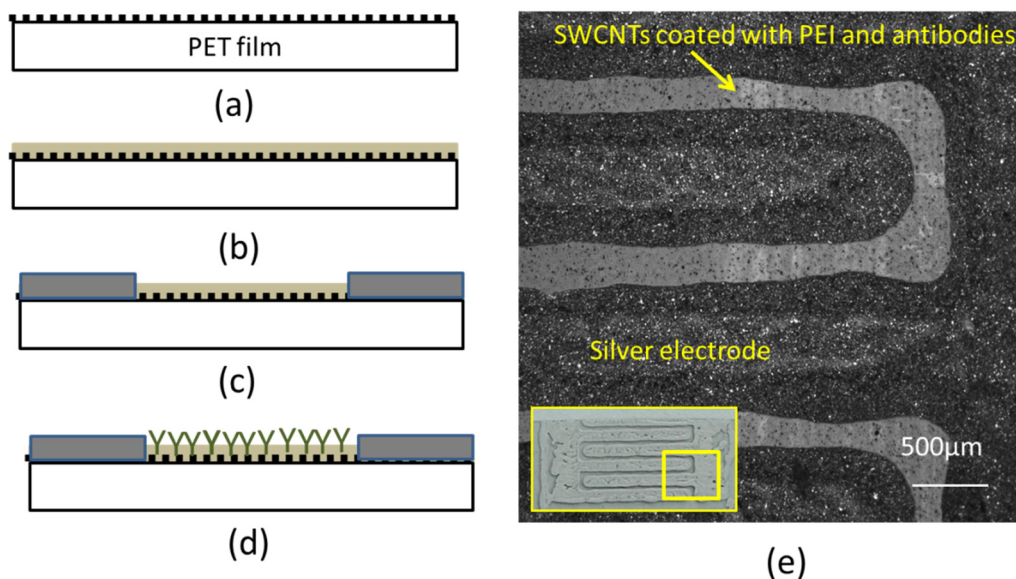


Fig. 12 Fabrication process of a SWCNT-based immunosensor (a) Spin coating of SWCNTs on a PET film (b) Spin coating of PEI (c) Stamping of silver electrodes. (d) Antibody immobilization (e) Photo and optical microscope images of a SWCNT immunosensor.

1.2. Preparation of magnetic particles:

Carboxyl functionalized superparamagnetic particles (Ocean Nanotech #MHP-100-01) were functionalized with anti-MPT64 antibody using a protocol modified from the bead manufacturer. Briefly, 600 μL aliquot of the magnetic particles (MPs) were washed by applying a magnet for 5 minutes and removing the storage solution. The bead solution was then re-suspended in a 0.5mL solution of 0.4M 1-ethyl-3-(3-dimethylaminepropyl) carbodiimide HCl (EDC) (Thermo Scientific # 22980) and 0.1M N-hydroxysulfosuccinimide (NHS) (thermos Scientific #24510) in DI water and incubated for 15 minutes. The activated beads were then washed once by magnetic separation

with 0.5 mL cold (4°C) DI water and then resuspended in 0.3 mL of the antibody solution [17mg/mL antibody in Dulbecco's Phosphate Buffered Saline (DPBS)] and reacted for 3 hours with mixing at room temperature. The bead-antibody solution was then washed 3 more times by magnetic separation in a storage buffer supplied by the manufacturer (10mM PBS buffer with 0.02% NaN_3 , 0.01 %Tween 20, and 0.1% BSA).

1.3. Resistive detection of targets in sputum samples using magnetic beads

Among the characterized sputum processing protocol, NaLc treated protocols were chosen to characterize the lower limit of detection (LLD). Sputum samples (100 μL) were mixed with 100 μL -NaLc and 100 μL -4%-SDS. MTB (100 μL) of each concentration was spiked in the mixture. The mixture was cured on a hot plate for 10 minutes at 60°C. Among the 400 μL sample, 200 μL was mixed with 10 μL of magnetic beads suspended in 1xPBS (450 μL). After 20 minutes of stirring, the magnetic beads were held by a magnet with gentle suction of the sample solution. 1mL of PBS was dispensed for washing the magnetic beads. After removal of the PBS rinsing solution by pipette, 500 μL of PBS solution was dispensed for suspension of magnetic beads bound with the target.

To evaluate LLD for MPT 64, the protocol was slightly modified. Sputum samples (100 μL) were mixed with 100 μL -NaLc and 100 μL -4%-SDS. MPT64 (100 μL) of each concentration was spiked in the mixture. Without 60°C curing to avoid protein damage, the mixture was mixed with magnetic beads. The resistance was measured before and after magnetic bead binding on SWCNT surface (Fig. 13).

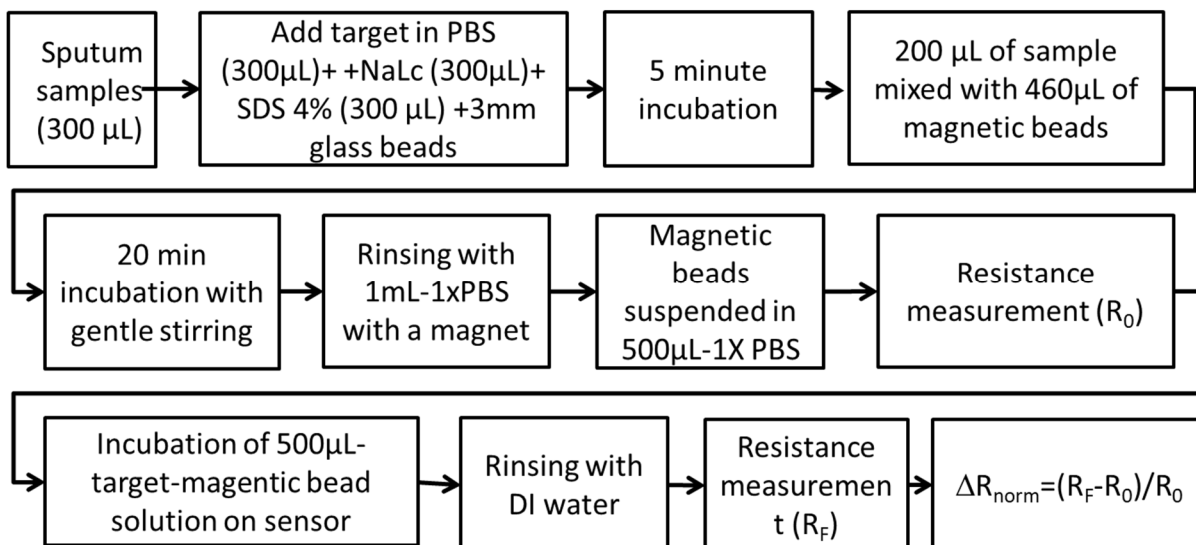


Fig. 13 Detection procedure of MTB (bacterial cells) and MPT64 (antigen) in sputum samples. Magnetic beads are used to capture and purify the target. The beads amplify the signal.

2. Results

To evaluate the LLD for sputum samples, MTB at various concentrations from 10^1 and 10^4 CFU/mL were spiked in sputum samples. The detection limit was 100 CFU/mL (Fig. 14a). According to the dose response test, the resistance change was not quantitative but qualitative. For the detection limit test using MTP-64 antigen, the LLD was 1 ng/mL, which was also qualitative (Fig. 14b). Given that sputum samples were replete with human cells, bacteria, and other microorganisms, these results also demonstrated the superior specificity of the SWCNT sensor using magnetic beads.

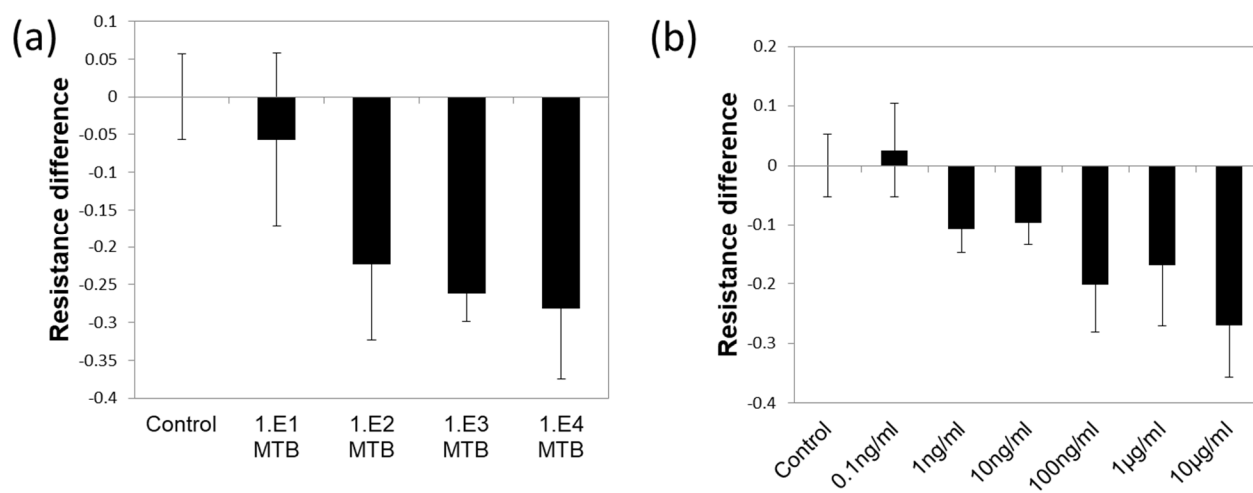


Fig. 14 Detection limit tests for MTB and MPT 64 (a) MTB spiked in sputum samples (b) MPT64 antigen in sputum samples.

Detection of target protein by applying magnets under the single walled carbon nanotube sensor. When a magnet is applied to a sensing platform, the resistance decreased with similar rate for control and target (MPT64 protein). When the magnet is removed from the sensor, the resistance of the control signal is recovered, but the resistance of the target is not fully recovered due to the presence of magnetic particles.

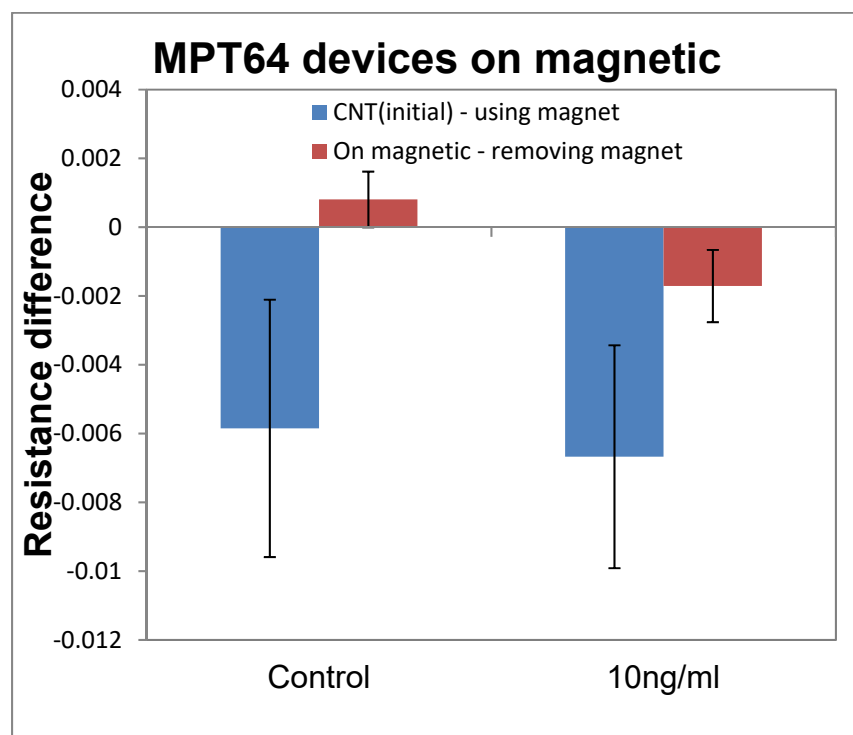


Fig. 15 Resistance change of a carbon nanotube sensor bound with magnetic particles for target and control.

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Carbon Nanotube-Based Thin-Film Resistive Sensor for Point-Of-Care Screening of Tuberculosis

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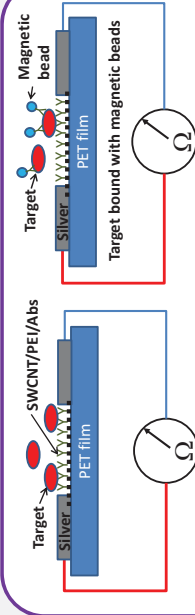
Introduction

- Tuberculosis, an infection caused by *Mycobacterium tuberculosis* (MTB), is one of the most serious infectious diseases worldwide.
- Although the incidence is gradually declining, developing countries have a significantly higher mortality rate than developed countries.
- For rapid TB screening, the collected samples are diagnosed with various methods, such as, the Ziehl-Neelsen (ZN) method for microscopic detection, immunoassays for antigen detection, or polymerase chain reaction (PCR) for DNA or RNA detection.
- The main challenge for TB diagnosis is the lack of rapid, simple, inexpensive, and accurate screening tools, especially for point-of-care (POC) diagnosis in resource-limited settings.
- Toward portable POC diagnostic systems, power source technology will need to meet the stringent requirements for safety, reliability, long-life, and high energy density.

Objectives

- The objective is to develop a resistive SWCNT biosensor on a polyethylene terephthalate (PET) film for low-cost TB screening. The developed protocol to functionalize single walled carbon nanotubes is evaluated for specific detection of targets (MTB and MPT64) from sputum and tongue swab samples.

Working Principle and Sensor Fabrication



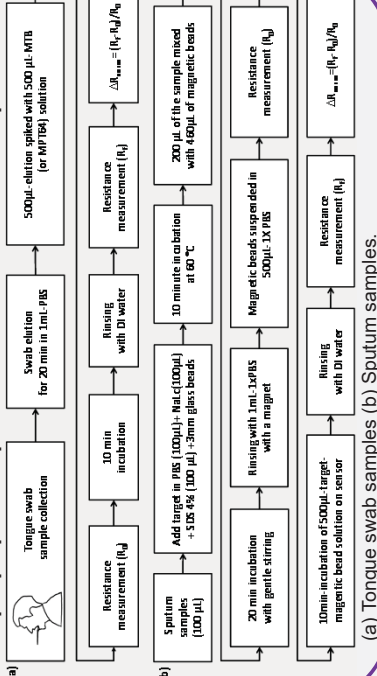
Cross-section of a resistive SWCNT immunosensor for direct target capture

- PET film
- Spin coating of SWCNTs
- Stamping of silver electrodes
- Antibody immobilization

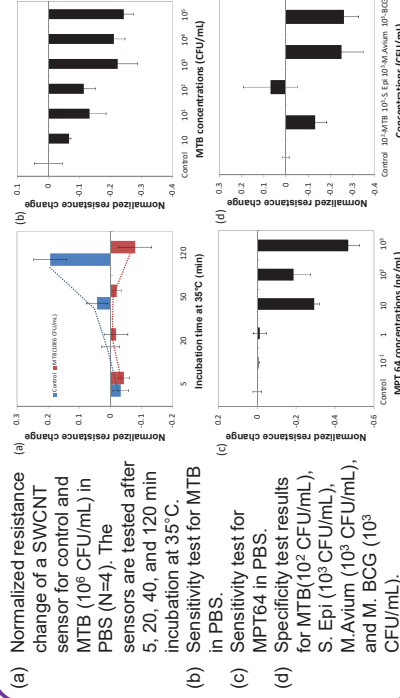
Fabrication process of a SWCNT-immunosensor

Experimental setup

Sample preparation protocol and resistive detection procedure



Experimental results – Antibody curing



Contact

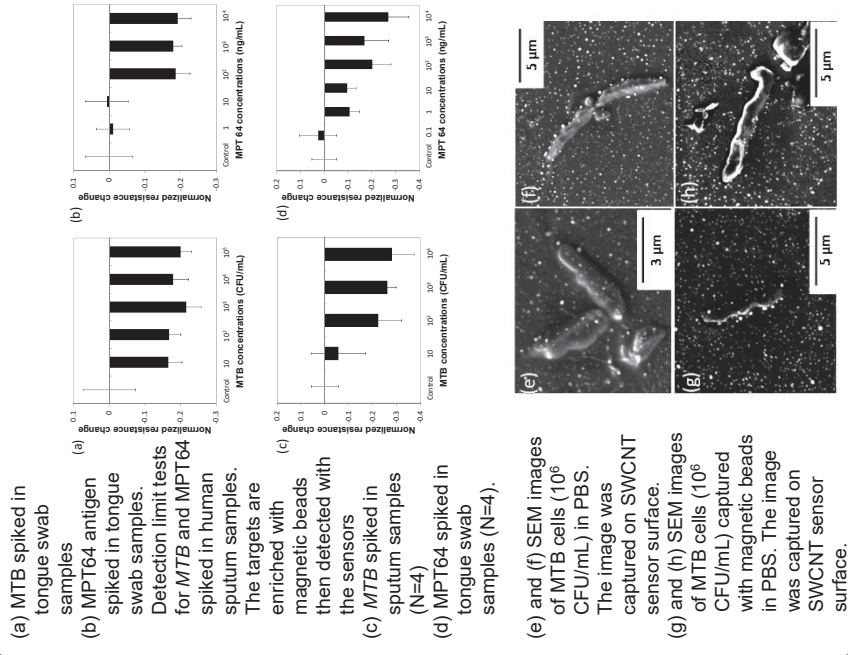
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Acknowledgement

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Experimental results

Detection limit tests for MTB and MPT 64



Conclusions

- The immuno-resistive SWCNT sensor was developed to specifically detect *Mycobacterium tuberculosis* (MTB) cells and surface antigen (MPT64) spiked in tongue swab and sputum samples.
- The detection limits were 10 CFU/mL for MTB and 100 ng/mL of MPT64 in tongue swab samples with the detection time of 30 minutes. For sputum samples, magnetic enrichment of targets was combined with the SWCNT sensors. The LLD for MTB and MPT64 spiked in sputa were 100 CFU/mL and 1ng/mL, respectively.

Carbon Nanotube-Based Thin-Film Resistive Sensor for Point-Of-Care Screening of Tuberculosis

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Abstract:

For point-of-care diagnosis of tuberculosis (TB), current TB diagnostic approaches need to be further improved for achieving an accurate diagnosis that is rapid and low-cost. This paper presents an immuno-resistive sensor on a plastic film for inexpensive, simple TB screening. The sensor is composed of single-walled carbon nanotubes (SWCNTs) functionalized with polyclonal antibodies raised against the MPT64 surface antigen from *Mycobacterium tuberculosis* (MTB). The target analyte consisting of either MTB or MPT64 is spiked in tongue swab and sputum samples. Unlike on atomically flat silicon chips, the major challenge for the development of a resistive SWCNT sensor on a plastic film is to achieve uniform performance on a rough polymer film. The fabrication and functionalization protocols of SWCNT sensors on a polyethylene terephthalate (PET) are developed for immuno-resistive detection of MTB and MPT64. Under optimized conditions, targets are directly detected from tongue swab samples. Target analytes spiked into the more complex matrix of human sputa are enriched with a magnetic bead protocol followed by detection with the SWCNT sensors. The sensitivity and specificity are determined along with the lower limit of detection in both samples. This highly

sensitive film sensor will facilitate inexpensive and rapid TB screening with the added benefits of a small form factor, simple operation, low power requirement, and low cost.

Key words: point-of-care diagnosis; tuberculosis; single-walled carbon nanotubes; resistive immunoassay; plastic film sensor

1. Introduction

Tuberculosis, an infection caused by *Mycobacterium tuberculosis* (MTB), is one of the most serious infectious diseases worldwide. Although the incidence is gradually declining, developing countries have a significantly higher mortality rate than developed countries (Sulis et al. 2016). In Asian and African countries, MTB infection occurs in 80% of the population (Gupta and Kakkar 2018). Currently, for the initial TB screening, three sputum samples are collected from a patient in the early morning. This sample collection procedure is then repeated several times for an initial diagnosis. Microbial culture from sputum is the gold standard diagnostic method, but requires laboratory infrastructure with trained personnel and takes a few weeks for results.

For rapid TB screening, the collected samples are diagnosed with various methods, such as, the Ziehl-Neelsen (ZN) method for microscopic detection, immunoassays for antigen detection (Sada et al. 1992), or polymerase chain reaction (PCR) for DNA or RNA detection (Dheda et al. 2013; Garcia-Basteiro et al. 2018). The ZN smear method is labor-intensive and not sufficiently sensitive for TB diagnosis (WHO 2008). Immunoassays, for example, the enzyme-linked immunosorbent assay (ELISA) for antigen detection, are rapid screening tools but with limited sensitivity and specificity (Sada et al. 1992). Among the screening approaches, PCR-based methods have shown clinical sensitivity and specificity greater than 95% with a 2 hour detection

time (Boehme et al. 2010). However, trained personnel in a well-equipped laboratory infrastructure are required with a stable electric power supply and relatively high running cost. Consequently, the main challenge for TB diagnosis is the lack of rapid, simple, inexpensive, and accurate screening tools, especially for point-of care (POC) diagnosis in resource-limited settings.

For use as a highly sensitive and specific screening tool for pathogen screening, nanomaterials have been investigated (Garcia-Basteiro et al. 2018). Among nanomaterials, single-walled carbon nanotubes (SWCNTs) are one of the potential candidates for enabling a simple resistive transducer to detect the binding of a target analyte with high sensitivity and specificity. The unique electronic properties (Barone et al. 2005; Chen et al. 2003; Cherukuri et al. 2004) render SWCNTs crucial to the development of inexpensive, sensitive biosensing platforms (Gruner 2006). The high sensitivity of a SWCNT biosensor stems from the small diameter (~ 1 nm) comparable to the size of a single biomolecule and the thickness of electrical double layers in physiological buffers (Maroto et al. 2007). In addition, the low charge carrier density of SWCNTs is comparable to the surface charge density of protein molecules and other antigens, which makes SWCNTs suitable for biomolecular detection (Heller et al. 2006). In comparison to optical and fluorescent detection, a resistive sensor operates with a simple measurement at low power in a small form factor.

Resistive SWCNT sensors can detect targets by two distinct mechanisms (Allen et al. 2007; Byon and Choi 2006; Heller et al. 2008; Li et al. 2005). One is to change the free carrier density of doped SWCNTs by electrostatic interaction. The other is to change the work function of the metal electrode-SWCNT interface, thus leading to Schottky barrier modulation. For SWCNTs deposited on gold electrodes on a silicon substrate, both mechanisms play roles in modulating the resistance. Viral particles and bacteria can be detected by measuring this resistance change

(Lee et al. 2011; Yoo et al. 2017). The lower limit of detection (LLD) of swine influenza virus (H1N1) was 177 TCID₅₀ (50% tissue culture infective dose)/mL (Lee et al. 2011). The LLD for *Bacillus subtilis* was 100 CFU/mL (Yoo et al. 2017). SWCNTs functionalized with heparin could detect dengue virus as low as 840 TCID₅₀/mL (Wasik et al. 2017). The LLD was 1 plaque forming unit (PFU)/mL for detecting H1N1 (Singh et al. 2014). Also, a similar sensing configuration was applied to detect a peanut allergen protein in food extracts with a detection limit of 5 ng/mL (Sobhan et al. 2018). In mRNA detection, the LLD was at attomolar levels, which showed the potential to detect nucleic acid without amplification. In our previous work, nanotips made of SWCNTs could be used for bacterial detection (Kim et al. 2013). The crossbar junctions coated with SWCNTs were fabricated to detect target bacteria in food samples at the detection limit of 100 CFU/mL (Kim et al. 2014).

Despite the great potential as a POC screening sensor, few resistive SWCNT biosensors have been demonstrated on flexible plastic films. Unlike atomically flat silicon substrates, a rough plastic film made of polymer renders the Schottky modulation unpredictable. The compatibility with silver electrodes, a popular conductive material on plastic films, significantly increases the contact resistance when SWCNTs are deposited on silver electrode surface (Kahng et al. 2018). The doping effect on SWCNTs by the plastic substrate, functionalization layers, and hydrogen binding in water-based buffer can also generate unreliable resistance changes. In comparison to the oxidation layer on a silicon chip, the charge of the SWCNTs is significantly changed by the plastic film. When SWCNTs are conjugated with antibodies in physiological buffer, hydrogen can be bound on SWCNTs to increase the resistance. As soon as the sensor is exposed to air out of buffer, the electrical resistance starts to decrease. The resistance change by target binding can

be interfered with hydrogen release, which can compromise the sensitivity and reliability of detection.

In this paper, a resistive SWCNT biosensor was fabricated on a polyethylene terephthalate (PET) film for low-cost TB screening. Silver electrodes were stamped on SWCNTs to reduce the contact resistance. The sensor response of SWCNTs coupled with silver electrodes was studied in conjunction with the binding of antibodies and target molecules. The sensitivity and specificity were characterized for *MTB* and surface antigen (MPT64) in phosphate buffered saline (PBS). The sensor was also characterized using two types of samples, tongue swabs and sputa. Oral swab samples were tested due to their recent discovery as a convenient biosample source for TB diagnosis (Luabeya et al. 2019; Wood et al. 2015). The targets in sputum samples were detected in combination with magnetic enrichment because of the sample complexity and the high ionic concentrations of reagents used in sputum liquefaction. The resistance change was measured upon the binding of either *MTB* or MPT64 spiked in two kinds of biosamples, tongue swab- and sputum samples. The presented biosensor will facilitate the development of a POC TB screening platform that has high sensitivity, low cost, and low power requirements.

2. Experimental method

2.1 Sensor fabrication

The sensors were fabricated on polyethylene terephthalate (PET) films (Fig. 1a). Target cells and antigen were detected using a SWCNT sensor functionalized with polyethyleneimine (PEI) and antibodies. Fig. 1b shows the direct detection of targets on the sensor surface, while Fig. 1c shows the detection of targets enriched with magnetic nanoparticles. Interdigitated

silver electrodes were stamped for resistive detection. When targets were bound on the sensor surface, the resistance decreased due to the electrostatic interaction.

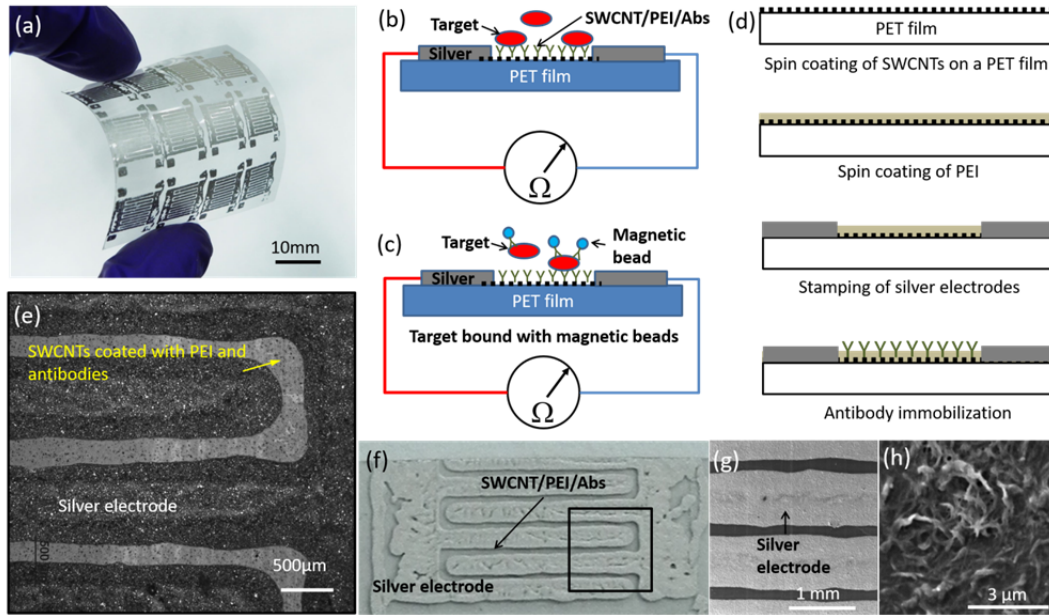


Fig. 1 (a) SWCNT-based sensor on a flexible PET film. (b) Cross-section of a resistive SWCNT immunosensor for direct target capture. (c) Cross-section of a resistive SWCNT immunosensor in combination with magnetic enrichment. (d) Fabrication process of a SWCNT-immunosensor (e) Optical microscope image of a SWCNT immunosensor. The dark region is silver electrodes. (f) Zoomed-out image of figure-e (g) SEM image. The bright area is silver electrodes. The dark area is SWCNTs (h) Exploded view of figure-g; a bundled SWCNT film.

For fabrication (Fig. 1d), SWCNTs were dispersed in 1%-SDS at a concentration of 5 mg/mL using an ultrasonic bath at room temperature for 3 hours. The SWCNTs were spin-coated onto a PET film at 6,000 rpm for 20 seconds. The SWCNT film was cured at 100°C on a hot plate for 10 minutes. PEI (0.1% in DI water) was coated on the SWCNT surface. Subsequently, the PEI-coated SWCNT film was cured at 100°C on a hot plate for 10 minutes. For silver electrode patterning, a Delrin[®] mold was machined by using an end mill. The stamp was made of polydimethylsiloxane (PDMS) cured in a mold at room temperature for 3 days. The PDMS

stamp coated with silver ink (EMS CI-1001) was used to print silver electrodes on the PEI-coated SWCNT sensors. The sensors were heated at 80°C on a hot plate for 1 hour to cure silver ink.

A polyclonal IgY antibody (1.8 mg/mL in PBS) raised against MPT64 protein was physisorbed on the SWCNT surface in PBS for 24 hours in a refrigerator (4 °C). Each sensor was cut with scissors by half to generate 2 sensors (Fig. 1e and 1f). A total of 24 sensors were fabricated on a 40×40 mm² PET film. Fig. 1f shows a sensor image composed of one pair of interdigitated electrodes. The silver electrodes having the gap size of 200~300 μm are connected with functionalized SWCNTs (Fig. 1g and 1h).

In the sensor configuration, silver electrodes were stamped on SWCNTs in order to minimize the exposure of the interfacial area between SWCNTs and silver electrodes. In the configuration, the oxidation of silver electrode surface should not affect the resistive change for target detection, which offered a uniform contact resistance and isolated the Schottky effect in the sensing mechanism. The electrostatic gating effect was the only mechanism that detected the target analytes.

2.2 Antibody preparation

Polyclonal IgY antibodies (pAb) were raised against purified MPT64 protein by Aves Labs (Davis, CA, USA). Complete Freund's adjuvant was used, thus, antibodies were reactive to *MTB* as well as MPT64. The antibodies were raised in two hens and evaluated by enzyme-linked immunosorbent assay (ELISA) to determine the binding to target MPT64 protein, and by filter plate enzyme immunoassay (EIA) to determine the reactivity to target cells.

To assay for the MPT64 protein, 100 μ L of a 100 μ g/mL solution of MPT64 in DPBS was added to an ELISA 96-well plate (Immulon 2HB, Thermo Scientific #3455). The mixture was incubated overnight at room temperature, followed by 1 hour incubation at 37 °C, and then washed with 3 $\times$ 200 μ L DPBS. To block the remaining sites in the well, a 200 μ L BSA solution in DPBS at 1 mg/mL was added and incubated for 1 hour at 37 °C followed by washing with 3 $\times$ 200 μ L DPBS. A 100 μ L solution of IgY (28 μ g/mL in DPBS) raised against MPT64 was added to each well. Control (pre-immune IgY) antibodies were tested at the same concentration. A 100 μ L solution of a 1:1000 dilution of secondary antibody (Rab anti-IgY-HRP Conjugate, Thermo Scientific #31401) was then added and incubated for 30 min at 37 °C, followed by DPBS washing (3 $\times$ 200 μ L). Finally, 100 μ L of ABTS substrate was added and measured at A₄₀₅ after 10 min incubation at room temperature. In comparison to pre-immune antibodies, the positive results were shown to MPT64 (Supplementary information, Fig. S1a).

To evaluate antibodies against *Mycobacterium*, the cultures of *Mycobacterium Bacillus Calmette–Guérin* (BCG) and *MTB* (H37Ra) cells were diluted to 1 $\times$ 10⁶ cells/mL in PBS, calculated by absorbance at OD₆₀₀, where the absorbance of 0.1 corresponded to a concentration of 6.3 $\times$ 10⁷ CFU/ml (Wayne 1976). The cell solutions (100 μ L of *MTB* or *BCG*) were then added to a well in a 96-well filter bottom plate (Millipore 0.45 μ M, #MAHVN4510). The cells were captured by filtration on the surface of the 0.45-micron filter and washed 3 times with 200 μ L Dulbecco's Phosphate Buffered Saline (DPBS) with vacuum filtration. A 100 μ L solution of IgY antibodies (28 μ g/mL) was added to each well and incubated for 30 min at 37 °C. Control (pre-immune IgY) antibodies were tested at the same concentration. The IgY solution was removed by vacuum filtration, and the filters were washed with 4 $\times$ 200 μ L DPBS. A 100 μ L solution of a secondary antibody (1:1000 dilution Rab anti-IgY-HRP Conjugate, Thermo Scientific #31401)

was added to each well and incubated for 30 min at 37°C, followed by washing with DPBS (3×200 µL). Finally, 100 µL 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) substrate (Thermo Scientific #37615) was added, followed by a 10 min incubation at room temperature. The solution was then filtered through the filter plate into a clear 96-well plate, which was read at A₄₀₅ in a microplate reader. According to the ELISA results, the polyclonal antibodies were specific to both *Mycobacterium* strains and non-tuberculosis *Mycobacterium* (NTM) species (Supplementary information, Fig. S1b)

2.3 Preparation of magnetic particles

Carboxyl-functionalized superparamagnetic particles (Ocean Nanotech #MHP-100-01) were functionalized with anti-MPT64 antibody using a protocol modified from the bead manufacturer. Briefly, a 600 µL aliquot of the 10mg/mL stock magnetic particles (MPs) was removed from the storage solution by applying a magnet for 5 minutes followed by careful removal of the storage liquid with a pipette. The bead solution was then resuspended in a 0.5 mL solution of 0.4 M 1-ethyl-3-(3-dimethylaminepropyl) carbodiimide HCl (EDC) (Thermo Scientific # 22980) and 0.1 M N-hydroxysulfosuccinimide (NHS) (Thermo Scientific #24510) in double-distilled (DDI) water and incubated for 15 minutes. The activated beads were then washed once by magnetic separation with 0.5 mL-DDI water (4°C), resuspended in 0.3 mL of the antibody solution (17 mg/mL antibody in DPBS), and reacted for 3 hours with mixing at room temperature. The bead-antibody solution was then washed three more times by magnetic separation in a storage buffer supplied by the manufacturer (10 mM PBS buffer with 0.02 % NaN₃, 0.01 % Tween 20, and 0.1 % BSA).

2.4 Sensor characterization

During antibody immobilization, the resistance of SWCNT sensors increased upon binding of antibodies, ions, and hydrogen on SWCNTs. When the sensor was exposed to air out of the antibody solution, the sensor resistance started to decrease due to the environmental change and hydrogen desorption. Of the attempts to increase the signal to noise ratio, the incubation at 35 °C after antibody immobilization was shown to produce the best result. The sensor response for the target samples (*MTB* at 10^6 CFU/mL in PBS) compared to control (no *MTB* in sample solution) was tested after 5, 20, 40 and 120 minutes of incubation at 35 °C. The curing temperature was not increased over 35°C to avoid degrading the antibodies. In the curing step, the humidity ranged between 25 and 30%.

2.5 Sensitivity and specificity tests

For sensitivity and specificity tests, both *MTB* and MPT64 were suspended in 1x PBS buffer. For *MTB*, various concentrations of *MTB* cells were suspended in PBS from 10^1 ~ 10^5 CFU/mL. MPT64 was also suspended in PBS from 0.1 ng/mL to 1µg/mL with 10 fold dilutions. 1 mL of each solution was supplied in each plastic cup where a sensor was immersed for immunocomplex formation. After 10 min of the incubation, the sensor was rinsed with DI water. After the gentle blow dry with nitrogen, the resistance was measured. The resistance values before and after immunocomplex formation were R_0 and R_f , respectively. The normalized resistance change $[(R_f - R_0)/R_0]$ was computed to compare the signal from the control.

For specificity tests, the response for *MTB* (10^2 CFU/mL) was compared with *Staphylococcus epidermidis* (*S. Epi* at 10^3 CFU/mL), *Mycobacterium Avium* (*M. Avium* at 10^3 CFU/mL), and *BCG* at 10^3 CFU/mL. The bacterial samples were suspended in 1 mL PBS.

2.6 Test using tongue swab samples

Tongue swab sampling is a newer approach for obtaining *MTB* markers of infection. To evaluate LLD for *MTB* and MPT-64 in tongue swab samples, the swab samples were prepared by scraping tongue surface from deidentified volunteers (Fig. 2a). After the complete drying of swabs in air, the swab samples were immersed in 1 mL PBS for 20 minutes with gentle stirring. Subsequently, 500 μ L of the target analyte (*MTB* or MPT64) in PBS was mixed with 500 μ L of the eluted swab solution. The 1 mL solution was used to test the LLD. The spiked concentrations of *MTB* ranged from 10 to 10^5 CFU/mL in steps of 10-fold dilutions. The concentrations of MPT64 ranged from 1 ng/mL to 10 μ g/mL with steps of 10-fold dilutions. For analysis, each sensor was incubated with a 1 mL sample solution for 10 minutes. Before and after target binding, the resistance was measured to compute a normalized resistance.

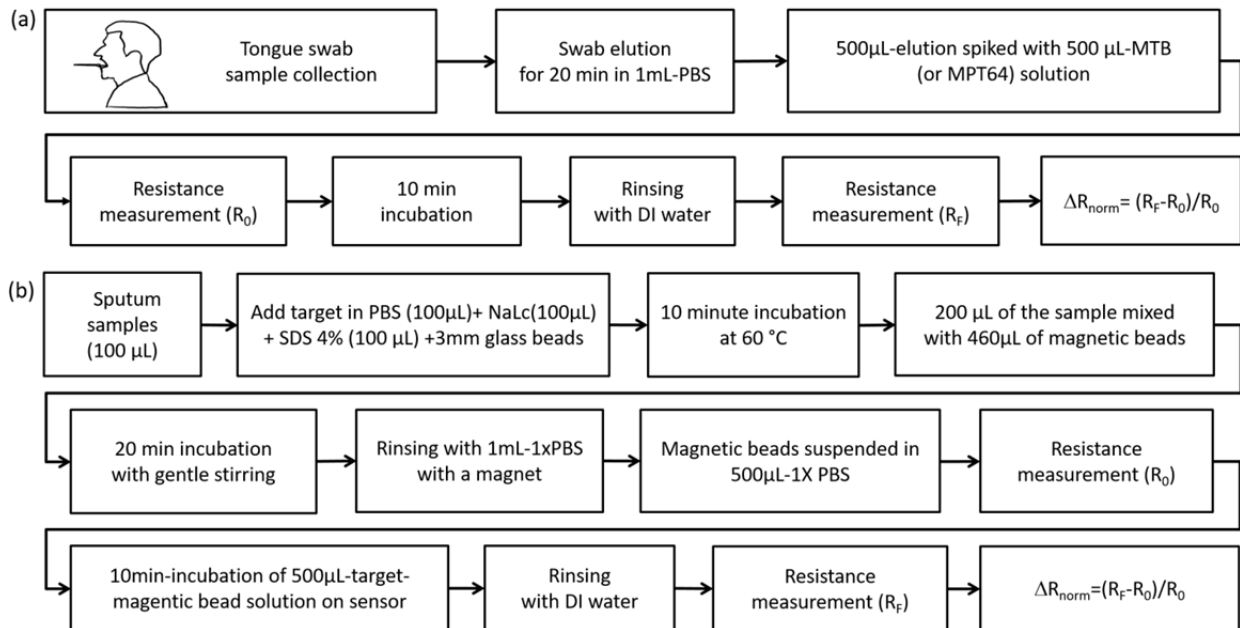


Fig. 2 Sample preparation protocol and resistive detection procedure (a) Tongue swab samples (b) Sputum samples.

2.7 Test using human sputum samples with magnetic nanoparticles

The test protocol for human sputum samples is described in Fig. 2b. Deidentified human sputum samples were obtained from BioReclamation, Inc. To reduce the viscosity and liquefy the sputum, 100 μ L sputum was first mixed with 100 μ L PBS followed by 100 μ L-NaLc (4 mg mL⁻¹N-acetyl-L-cysteine). Also, 3 mm-glass beads and a 4% SDS solution (sodium dodecyl sulfate, 100 μ L) were added to the mixture with the addition of the targets. The mixture was vortexed for 10 minutes with 60°C heating for complete liquefaction. This protocol was developed in our previous study (Kim et al. 2012).

For magnetic enrichment of *MTB*, 200 μ L of the 400 μ L-liquefied sputum samples were mixed with 10 μ L of magnetic beads suspended in 450 μ L PBS. After 20 minutes of gentle stirring and incubation, the magnetic beads were held with a magnet while the sample solution was gently aspirated. The magnetic beads were then washed with 1 mL PBS followed by magnetic separation. After rinsing, 500 μ L of PBS solution was used to suspend the magnetic beads bound to the target. Using this protocol, the LLD was evaluated for *MTB*.

To evaluate LLD for MPT64, the protocol was slightly modified. Sputum samples (100 μ L) were mixed with 100 μ L-NaLc and 100 μ L-4 %-SDS. MPT64 (100 μ L; 0.1~10⁴ ng/mL in 10-fold increments) of each concentration were spiked in the mixture. Without 60 °C heating to avoid protein damage, the dissipated sputum samples were mixed with the magnetic beads. The following procedure was the same as the *MTB*-sputum protocol.

3. Experimental Results

3.1 Sensor characterization

In the 24-hour antibody immobilization step, the sensor resistance was increased by the binding of hydrogen, ions, and antibodies. In the resistance measurement using various antibody concentrations in the immobilization step, the 65 % of the resistance change was attributed to hydrogen and ion bonding, and 35 % was from antibody binding (Supplementary information, Fig. S2). To study the curing effect of a 35 °C step following antibody immobilization, the immunoassay using SWCNT sensors was tested after 5, 20, 40, and 120 minutes of incubation at 35 °C. Fig. 3a shows the normalized resistance change for *MTB* (10^6 CFU/mL) at 35 °C.

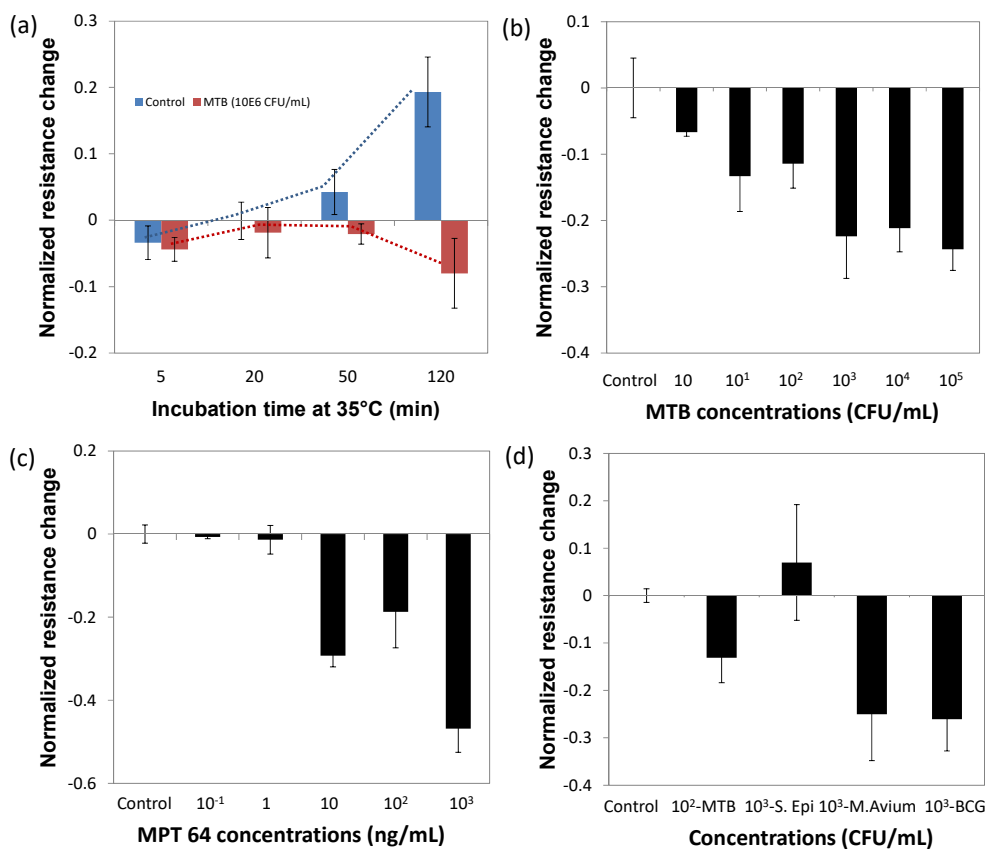


Fig. 3 (a) Normalized resistance change of a SWCNT sensor for control and MTB (10^6 CFU/mL) in PBS (N=4). The sensors are tested after 5, 20, 40, and 120 min incubation at 35°C. (b) Sensitivity test for MTB in PBS. (c) Sensitivity test for MPT64 in PBS. (d) Specificity test results for MTB (10^2 CFU/mL), S. Epi (10^3 CFU/mL), M. Avium (10^3 CFU/mL), and M. BCG (10^3 CFU/mL).

The control was negative at 5 min and gradually increased to the positive value. The normalized resistance of the positive *MTB* samples maintained slightly negative values and dropped to -0.08 on average. When the control was compared with the positive cases, a signal could be differentiated for the samples of 40 and 120 min incubations at 35 °C. In the following sensitivity, specificity, and LLD experiments, the incubation time and temperature were maintained as 120 min and 35 °C, respectively. The incubation condition provided a consistent increase of the resistance values (Supplementary information; Fig. S3).

3.2 Sensitivity and specificity tests

For sensitivity tests, various concentrations of *MTB* cells in PBS buffer were tested, as shown in Fig. 3b. The signal from 10 to 10⁵ CFU/mL was compared to the control. In these tests, the normalized resistance change for the control was measured between 0.15 and 0.25. The average value of the normalized resistance for the control was shifted to 0 for convenience of reporting. The control signal was shifted down, while the detection signal was even further decreased. Despite the high sensitivity, the resistance change was not quantitative with respect to *MTB* concentration. When the dose-response test was conducted for antigen MPT64 (Fig. 3c), the signal was detectable starting at 10 ng/mL.

For the specificity test, the signal of *MTB* at 100 CFU/mL was clearly differentiated from the control and *S. epi* at 10³ CFU/mL (Fig. 3d). However, *M. Avium* (10³ CFU/mL) and *BCG* (10³ CFU/mL) showed a positive response due to the cross-reactivity to *Mycobacterium* strains, including NTM. The cross-reactivity to *Mycobacterium* strains corresponded with the results of the ELISA assay (Supplementary information, Fig. S1b).

3.3 Tests using tongue swab samples and human sputum samples with magnetic beads

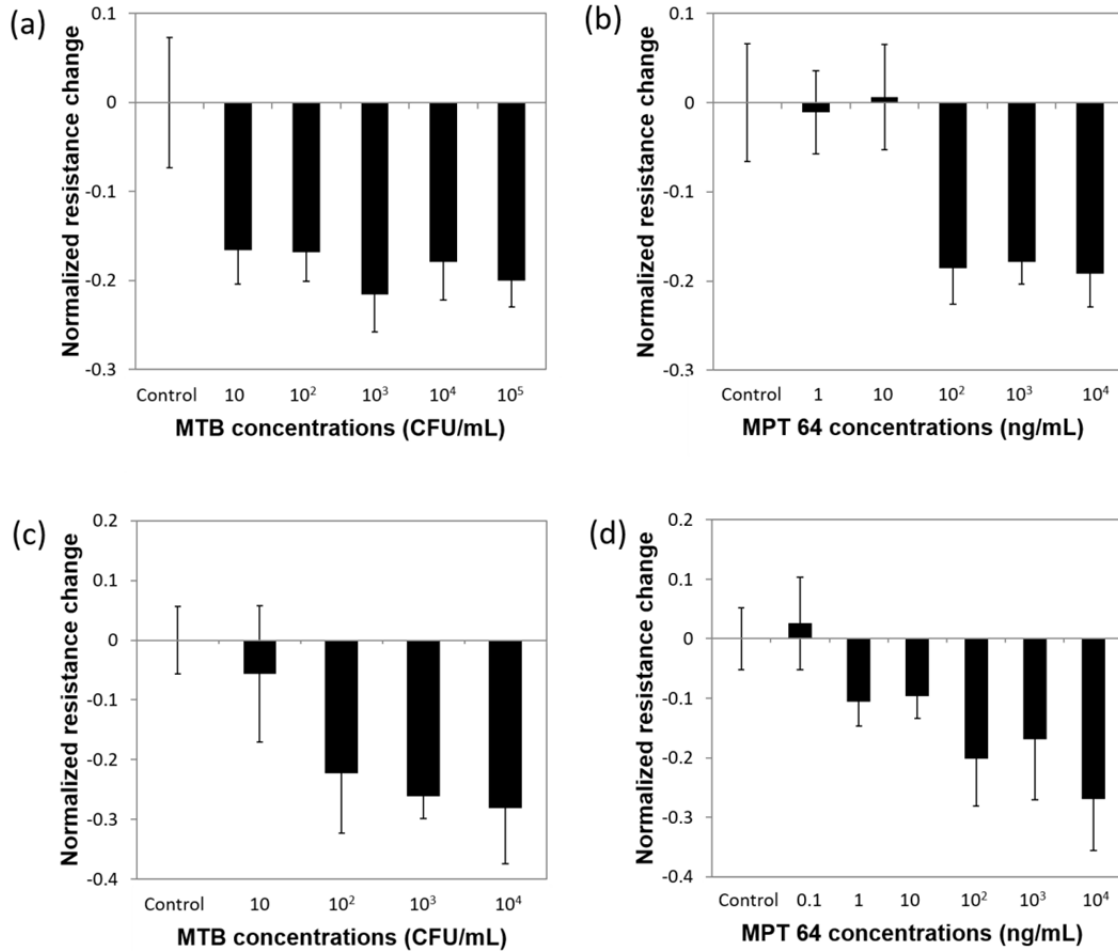


Fig. 4 Detection limit tests for MTB and MPT64 (a) MTB spiked in tongue swab samples (b) MPT64 antigen spiked in tongue swab samples. (c) MTB spiked in sputum samples (N=4). The targets are enriched with magnetic beads then detected with the sensors. (d) MPT64 spiked in tongue swab samples (N=4). The targets are enriched with magnetic beads then detected with the sensors.

To evaluate the LLD for tongue swab samples, *MTB* at the concentrations ranging from 10 to 10⁵ CFU/mL were spiked into tongue swab samples. The detection limit was 10 CFU/mL (Fig. 4a). According to the dose-response test, the resistance change was not quantitative but qualitative. For the detection limit test using MPT64 antigen, the LLD was 100 ng/mL, which

was also qualitative (Fig. 4b). Given that tongue swab samples were replete with human cells, bacteria, and other microorganisms, these results also demonstrated the superior specificity of the SWCNT sensor.

For the LLD for human sputum samples, *MTB* cells of $10 \sim 10^4$ CFU/mL were mixed with NaLc-treated sputum samples. MPT64 was spiked in the range of $0.1 \sim 10^4$ ng/mL. The detection limit was 10^2 CFU/mL (Fig. 4c) for *MTB* and 1 ng/mL for the MPT64 antigen (Fig. 4d).

To validate if the target cells were captured on a sensor surface, *MTB* cells (10^6 CFU/mL in PBS) were observed on the SWCNT surface (Fig. 5a and 5b). Fig. 5(c) and 5(d) show the SEM images of *MTB* cells (10^6 CFU/mL in PBS) bound with magnetic beads on the SWCNT surface. In the images, the white dots appeared crystallized ions from PBS. Under the SEM images, magnetic nanoparticles could not be discerned from the crystal ions. The qualitative, not quantitative signal could be caused by the binding nature between bacterial cells and sensor surface. Considering the effective range of electrostatic detection as 10 nm, the nonuniform binding of target cells could result in a qualitative signal. The qualitative signal may also be related to the large gap size of 200 μm , explaining the saturation of the resistance change in the large gap size (Lee et al. 2011).

The use of PET films as sensor substrates can significantly reduce the material and manufacturing costs. Unlike the gold electrodes on silicon chips, the deposition of SWCNTs on silver electrodes resulted in unreliable contact resistance due to the oxidized silver layer. The rough surface of a PET film made the contact resistance higher (Kahng et al. 2018). According to our study using an atomic force microscope, the roughness ranges from 15 to 80 nm with the bumps on the surface (Supplementary information; Fig. S4). By stamping silver electrodes on a SWCNT film, a reliable resistance of a SWCNT sensor could be obtained. One of the major

differences between silicon and PET substrates was the doping of SWCNTs on the PET film. While the SWCNTs on oxidized silicon chips were doped with hydroxyl groups, those on PET films were doped with carboxyl groups. Although both substrates made SWCNTs p-type, the doping on a rough PET film could significantly change the contact resistance of a SWCNT sensor in combination with the PEI layer. For stable performance, the delicate control of the functionalization layers was critical. In future, the addition of a control sensor next to a sensor will enhance the signal-to-noise ratio by compensating environmental factors including temperature.

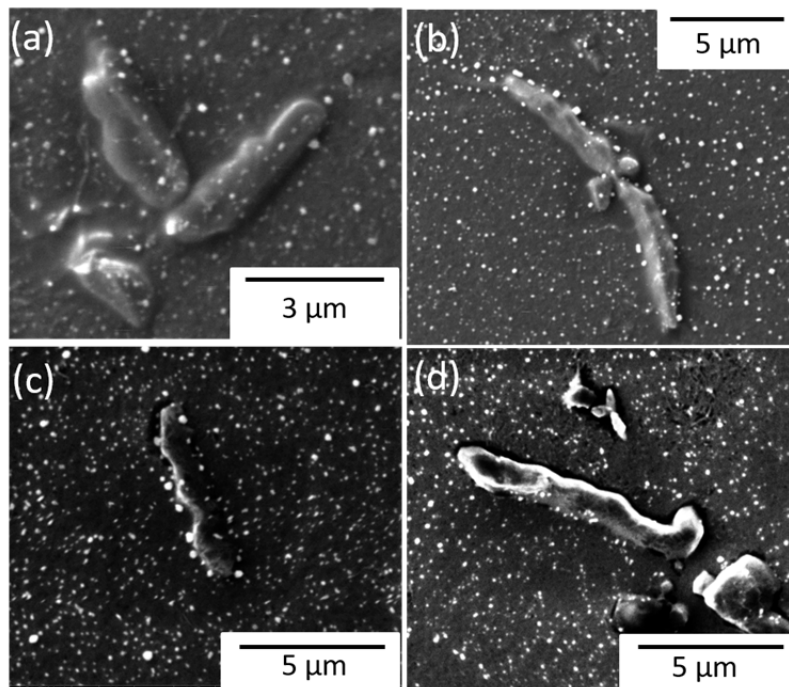


Fig. 5 (a) and (b) SEM images of MTB cells (10^6 CFU/mL) in PBS. The image was captured on SWCNT sensor surface. (c) and (d) SEM images of MTB cells (10^6 CFU/mL) captured with magnetic beads in PBS. The image was captured on SWCNT sensor surface.

Regarding the curing effect on the sensor performance, the role of the PEI layer on the SWCNT film may be critical for improving the signal to noise ratio. In the additional tests to

observe the resistance change, the SWCNT sensors were incubated in the antibody solution, PBS, and DI water. The incubated samples were cured at 25 and 35°C for 5 hours. As the incubation time increased, the resistance decreased due to hydrogen desorption (Supplementary information; Fig. S5a). Interestingly, the resistance change at 35°C was smaller than 25°C. We speculated that the resistance change was also related to the oxidation of the PEI layer. With the greater oxidation of the amine group of the PEI layer at the higher temperature, the decrease of the SWCNT resistance at 35°C became lower than that at 25°C. Without a PEI layer on SWCNTs, the resistances of SWCNTs immersed in DI water and cured at both 25 and 35 °C were almost identical (Supplementary information; Fig. S5b). The bump of the resistance in the air exposure was caused by the counter doping effect of CNTs in water (Han et al. 2012; Zhang et al. 2019). According to the results, the delicate control of SWCNT doping with a PET film, a PEI layer, antibodies and air could be a key to the successful development of a resistive plastic film sensor. The test results need to be considered for packaging the sensors for longer shelf life.

The flexible PET film substrate can be attached or bent to fit a testing condition. The resistance change upon bending was tested (Supplementary information; Fig. S6). When the sensor was bent by a radius of 1.5 mm and recovered with the stress release, the resistance change was 0.33 %. In comparison to the change of signal resistance > 10% with specific measurements, the 0.33 % resistance change can be neglected. The bending test results show that the sensor can be bent during target binding and operation. However, the measurements should be conducted without external stress. The flexible nature will benefit the sensor application in the platforms requiring a small form factor and low cost.

4. Conclusion

In summary, the immuno-resistive SWCNT sensor was developed to specifically detect *Mycobacterium tuberculosis* (MTB) cells and surface antigen (MPT64) spiked in tongue swab and sputum samples. The detection limits were 10 CFU/mL for MTB and 100 ng/mL of MPT64 in tongue swab samples with the detection time of 30 minutes. For sputum samples, magnetic enrichment of targets was combined with the SWCNT sensors. The LLD for MTB and MPT64 spiked in sputa were 100 CFU/mL and 1ng/mL, respectively. The LLD was comparable to PCR but without requiring bacteriological culture, centrifugation, or nucleic acid amplification. To achieve such high sensitivity and specificity, the resistance change of a SWCNT sensor coupled with the fabrication and functionalization protocols was studied to determine the optimal curing temperature and time of 35°C and 2 hours. Unlike other SWCNT-based sensors employing silicon chips, the presented sensor was fabricated on a flexible PET film, which will potentially provide a low cost and a lightweight platform. The simple resistive measurement will allow rapid screening by minimally trained personnel. Also, a minimal power requirement (<1 W) combined with low assay cost will be ideal for point-of-care (POC) screening in limited-resource settings.

Conflicts of interest

There are no conflicts to declare.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary information

Carbon Nanotube-Based Thin-Film Resistive Sensor for Point-Of-Care Screening of Tuberculosis

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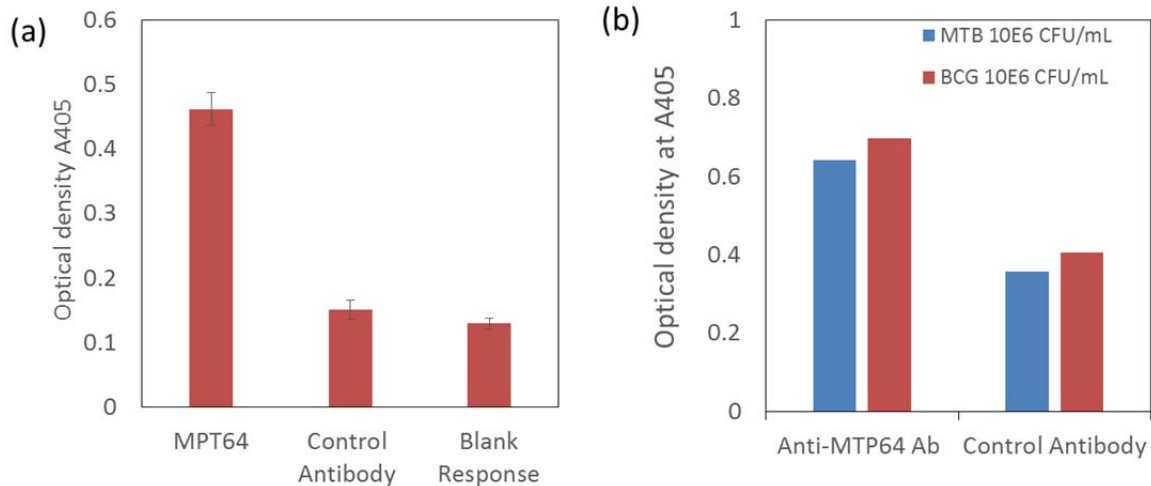


Fig. S1 (a) Optical density showing the binding of MPT64 antibodies (28 µg/mL) to MTB (10⁶ CFU/mL) and BCG (10⁶ CFU/mL). (b) Optical density showing the binding of MPT64-antibodies to MPT64 in comparison to control.

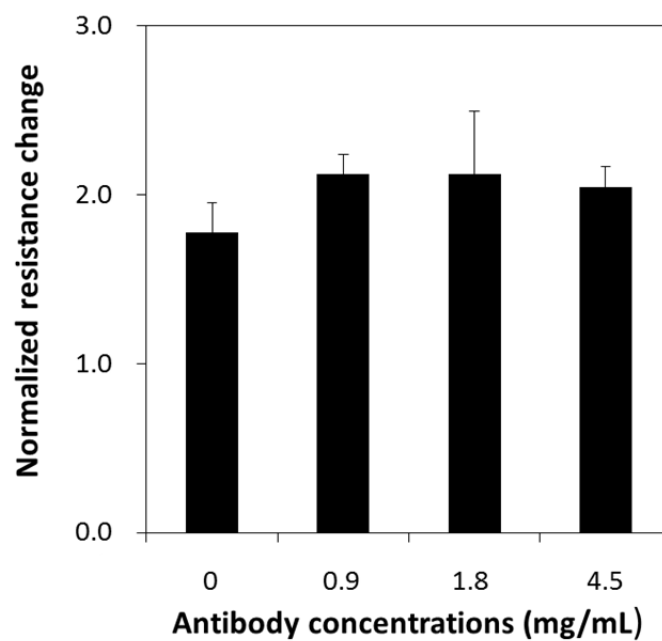


Fig. S2 Normalized resistance change with immobilizing antibodies of various concentrations on SWCNT sensors (N=4).

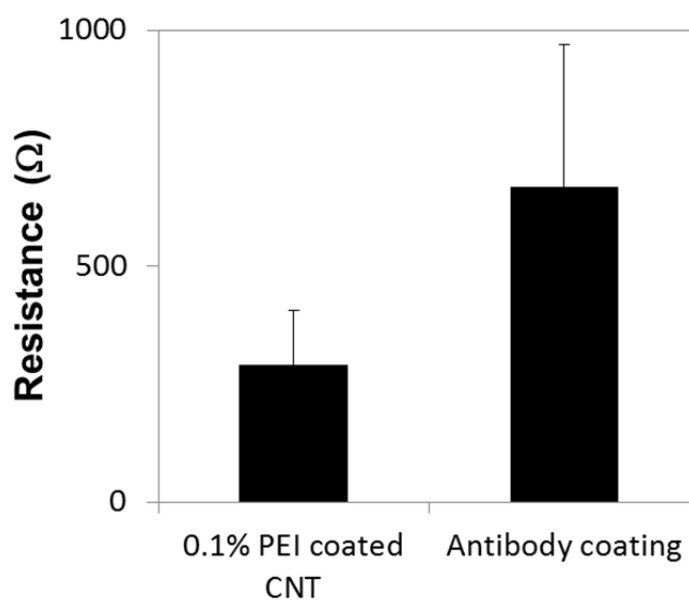


Fig. S3 (a) Normalized resistance change before and after antibody immobilization on SWCNT sensors (N=4).

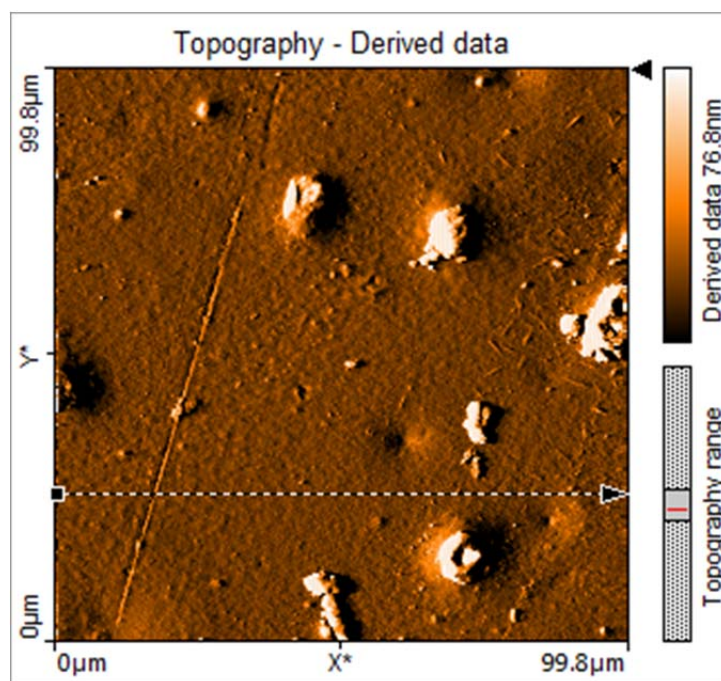


Fig. S4 AFM image of a PET film used in the experiment. The roughness is smaller than 80 nm.

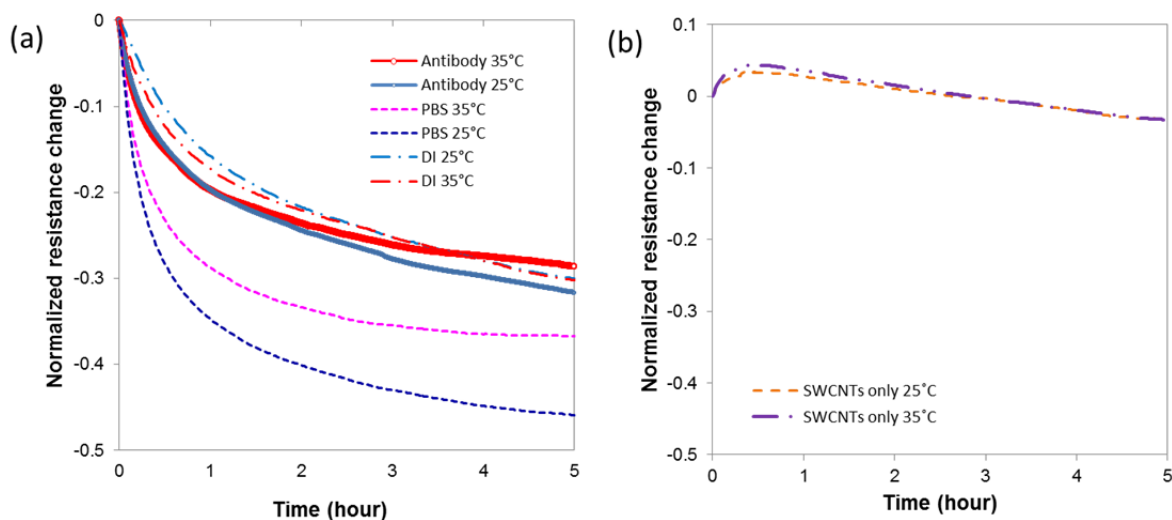


Fig. S5 (a) Normalized resistance change of a SWCNT immunosensor at 35 °C after antibody immobilization. The sensor is coated with PEI before antibody immobilization. (b) Normalized resistance change of a SWCNT sensor at 25 °C and 35 °C after immersion in DI water for 24 hours. The SWCNT sensor is not coated with PEI and antibodies.

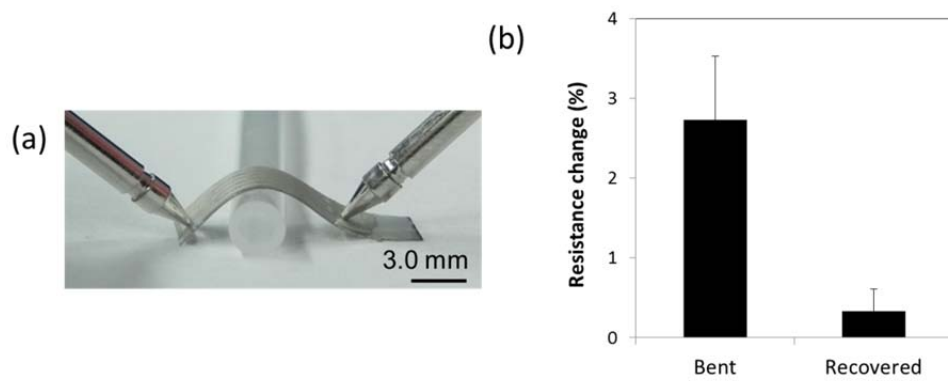


Fig. S6 (a) Bending test using 3 mm silicone bar (b) Resistance change for the 1st bending and the 1st recovery (N=6).

Highlights

1. For point-of-care (POC) diagnosis of tuberculosis (TB), an immuno-resistive single-walled carbon nanotube (SWCNT) sensor in combination with magnetic enrichment was developed on a flexible PET film substrate for the first time.
2. The fabrication and functionalization protocols on a rough PET film surface were developed to address the issues of contact resistance and ionic bonding.
3. The immuno-resistive SWCNT sensor was evaluated for tongue swab and sputum samples spiked with *Mycobacterium tuberculosis* (MTB) cells and antigen (MPT64), which showed one of the lowest detection limits among immunosensors.
4. The SWCNTs immuno-resistive sensor will facilitate a low cost, light weight, low power and rapid screening sensor.

Credit Author Statement

Seong-Joong Kahng fabricated, tested, and analyzed the experimental results. Based on the analysis, the draft of the manuscript was prepared. **Scott D. Soelberg** designed the bioassays, prepared and evaluated biomolecules and magnetic nanoparticles, and edited the manuscript. **Fabrice Fondjo** fabricated and tested the biosensors for MPT64. **Jong-Hoon Kim** designed and analyzed the experimental results. The manuscript was edited. **Clement E. Furlong** designed biomolecules and bioassays and edited the manuscript. **Jae-Hyun Chung** initialized the sensor concept, led the sensor development, and edited the manuscript.