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TITLE: Nanoink Printed Amperometric Immunosensor for Rapid and Inexpensive Screening of Tuberculosis

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| <b>13. SUPPLEMENTARY NOTES</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                        |                                         |                                                       |                                                     |                                                   |
| <b>14. ABSTRACT</b><br>The project aims to develop a point-of-care diagnostic platform for tuberculosis diagnosis. The sensor detects Mycobacterium tuberculosis (MTB; H37Ra strain) cells and MPT-64 antigen spiked in human sputum samples. Single walled carbon nanotubes are used as a sensing element for detection of target analytes. In the project period, various SWCNTs-based immunosensors were designed and fabricated. The fabrication and detection protocol was optimized. For specific detection, antibodies to MPT-64 were generated and characterized. Through affinity purification, the antibodies became more sensitive and specific to target analytes. Using the sensor, the detection limit was $10^6$ CFU/mL for BCG and 100 ng/mL for MPT-64. In the next project period, the detection protocol will be further optimized to achieve detection limit of $10^3$ CFU/mL (or equivalent concentration to MPT-64). |                                        |                                         |                                                       |                                                     |                                                   |
| <b>15. SUBJECT TERMS</b><br>Tuberculosis, Carbon nanotubes, Point-of-care diagnosis                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                        |                                         |                                                       |                                                     |                                                   |
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1. **INTRODUCTION:** (subject, purpose and scope)

Subject: The project is to develop a point-of-care diagnostic platform for tuberculosis diagnosis. The sensor identifies Mycobacterium tuberculosis (MTB) cells (H37Ra strain) and MPT-64 antigen spiked in human sputum samples. The film-type amperometric immunosensor detects the target analytes by using single walled carbon nanotubes (SWCNTs) printed on a plastic film. Upon binding of target to sensor surface, the electric current is changed to identify target analytes-MTB cells and MPT-64 spiked in human sputum samples. To enhance the specificity, antibodies against MPT-64 are raised and tested to identify TB in sputum samples. In addition, the project involves the optimization of the processing of human sputum samples prior to detection with the biosensor.

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

Scope: In the project, we aim 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. The sample is benign sputum samples spiked with MTB and MPT-64. Upon binding, the electric current through SWCNTs is changed to detect target analytes without culture or PCR amplification. In the first year, the sensor is optimized to detect target analytes spiked in phosphate buffer saline buffer (PBS). Note that the sensor is optimized with BCG and MPT-64 in the first year. BCG is replaced with MTB in the 2<sup>nd</sup> year. Antibodies are raised against MPT-64 to evaluate the specificity. In the 2<sup>nd</sup> year, the sensor is evaluated for sputum samples spiked with MTB and MPT-64.

2. **KEYWORDS:** MTB, BCG, MPT-64, IgY, Carbon nanotubes, Printing, Diagnosis, Immunoassay.

### 3. ACCOMPLISHMENTS:

**The major goals and the planned dates in the SOW are described as below.**

**A. Design and fabricate SWCNTs-based immunosensors (Planned dates: May 15, 2017).**

A1: Fabrication of various dimensions of SWCNT based sensors

A2: Study of SWCNTs doped with various concentrations of PEI

**B. Sensor optimization for specificity and sensitivity using pure samples (Planned dates: September 15, 2017).**

B1: Preparation and evaluation of antibodies for BCG and MPT-64

B2: Fabrication of antibody immobilized sensors

B3: Evaluation of sensor to sensor variation.

**C. Demonstrate the prototype device with pure samples (Planned dates: March 15, 2017).**

C1: Preparation of cells and MPT-64

C2: Demonstration of sensor performance for MTB (BCG) in PBS buffer.

C3: Demonstration of sensor performance for MPT64 in PBS buffer.

**The accomplishment and the actual completion dates are described as below.**

A rapid and simple method for TB screening was developed using nanoink printed sensors functionalized with polyethylenimine (PEI), single-walled carbon nanotubes (SWCNTs) and antibodies. The novelty of this biosensor is based on its simplicity and low production cost. The stamping of silver ink defined an array of electrodes on a polyethylene terephthalate (PET) film. An area of the silver electrodes was functionalized with PEI, SWCNTs and antibodies sequentially. PEI coating enabled SWCNT doping for electrical detection and ionic binding of antibodies for immobilization. To characterize and evaluate the fabricated sensor performance, the following tests have been conducted:

**A. Design and fabrication of SWCNTs-based immunosensors (Completed dates: March 15, 2018).**

A1: Fabrication of antibody-immobilized sensors with various electrode configurations to improve the signal-to-noise ratio.

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.

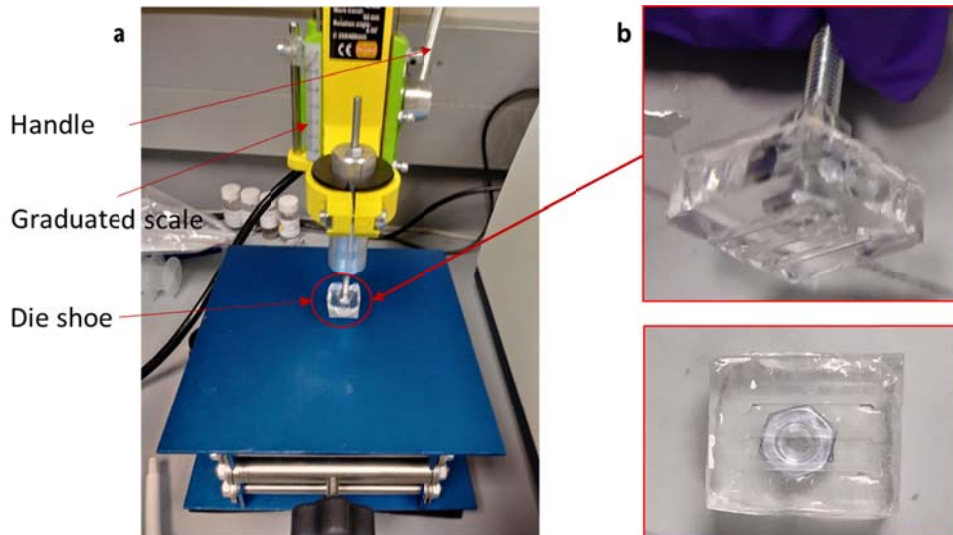


**Figure 1.** Stamping setup. (a) Stage of the stamping process (b) the stamp made of PDMS. Two setups were constructed, one for Chung's group at University of Washington and a second for Kim's group at Washington State University.

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.

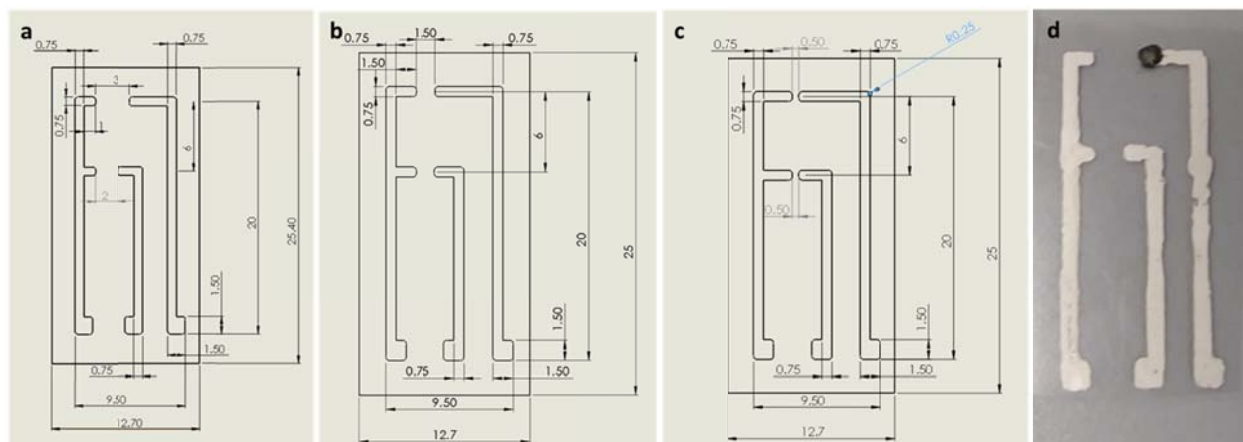


**Figure 2.** Various electrode configurations. (a) 3 and 2mm gap device. (b) 1.5mm gap device. (c) 0.5mm gap device. (d) Fabricated device

#### A2: 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 ( $\theta_{B,a}$ ) 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,  $\theta_{B,a}$  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 the surface increases to reduce the ink flow. For low  $\theta_{B,a}$ , the pressure difference is maximized resulting in the high ink feed rate.

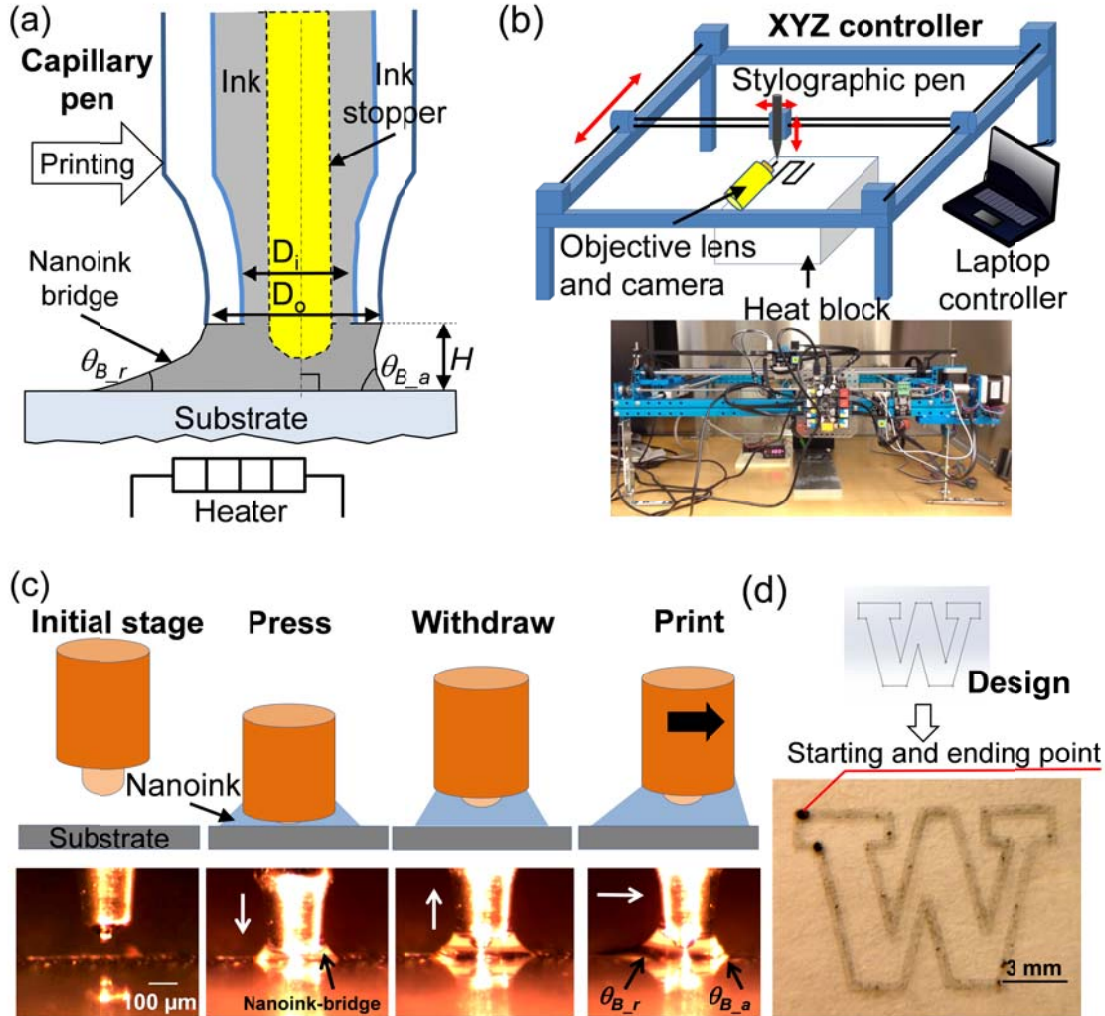


**Figure 3.** Nanoink bridge induced capillary printing (a) Printing concept (b) Schematic of an xyz plotter installed with a heating stage and a camera system. The top image shows a printing system, and the bottom is a photograph of the setup. (c) Nanoink-bridge induced printing using water ink on a PET film. The ink is released by pressing the stopper. Upon withdrawal by 100  $\mu\text{m}$ , 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°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  $H=100\text{ }\mu\text{m}$ ; a 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. 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 (accepted 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  $\mu\text{L}$ ) were evaluated based on the electrodes.

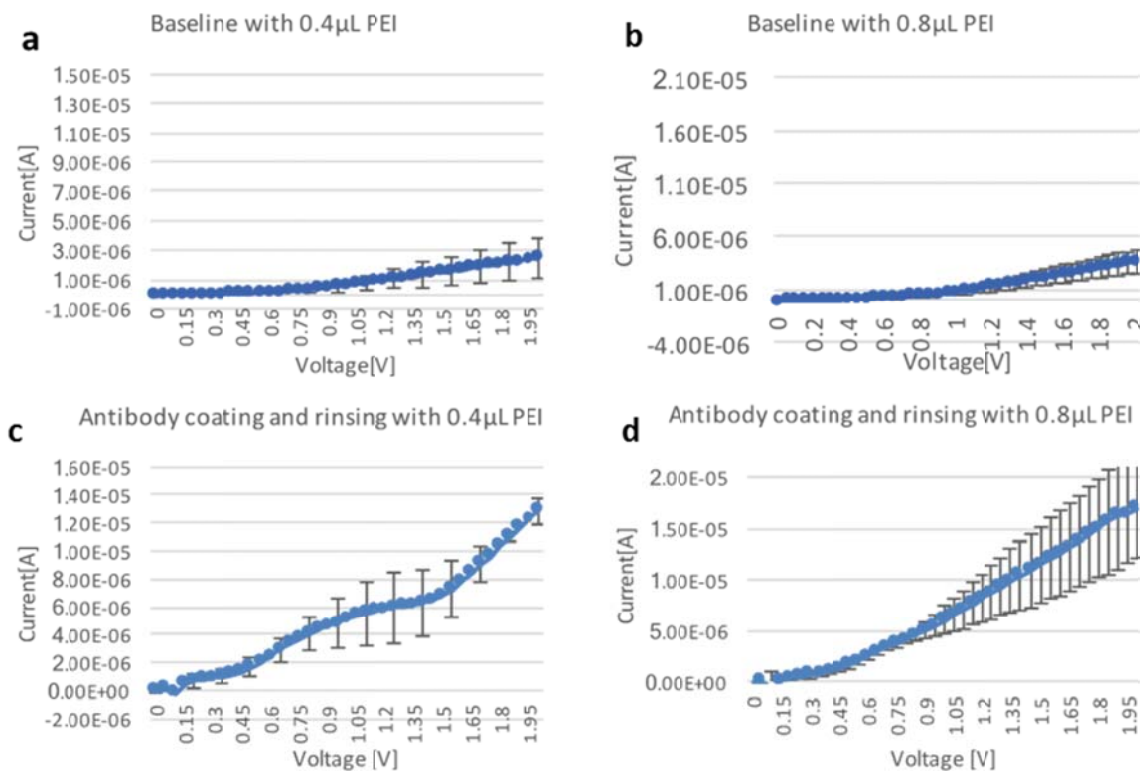


**Figure 4.** I-V curves for 1% PEI/SWCNTs in SDS coatings. (a) Baseline with 0.4 $\mu\text{L}$  of 1% PEI and (b) 0.8 $\mu\text{L}$  of 1% PEI, (c) I-V curve after antibody coating and rinsing with a 0.4 $\mu\text{L}$  PEI layer and (d) with a 0.8 $\mu\text{L}$  PEI layer.

SWCNTs were dispersed in SDS 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, 0.5µl 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 ~100 KΩ. 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 0.4µl PEI coating after antibody coating and rinsing showed a smaller error bar around half of the 0.8µL PEI, meaning that 0.4µl PEI layer generated more consistent current. This led us to proceed with the smaller volume of PEI coating (0.4µL PEI).

Final procedure for sensor fabrication is as below:

1. With the silver ink stamped and cured on the PET film, the film is set on a hot plate at 60°C, the sensing area of the device was first covered with a 1µl of PEI.
2. The temperature was increased to 100°C to allow the PEI to be fully cured for 10 minutes.
3. Subsequently, the device was set on a hot plate at 80°C and 0.5µl of SWCNT in DMF was printed on top of the cured PEI film and let to cure for 10 minutes. This allowed a uniform deposition of the CNT on the sensing area and the complete evaporation of DMF.
4. Three additional layers of SWCNTs were added on top of one another to increase the resistance of the sensing area to 100 KΩ.
5. Finally, a well was installed on top of the sensing area of the device using Teflon® tape punched with a 3mm diameter hole to ensure that none of the testing fluids flowed away from the intended area (sensing area).

## **B. Sensor optimization for specificity and sensitivity using pure samples (Completed dates: March 15, 2018).**

### B1. Preparation and evaluation of antibodies for BCG and MPT64

In this task, BCG cells and MPT64 protein were prepared. The previously 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.

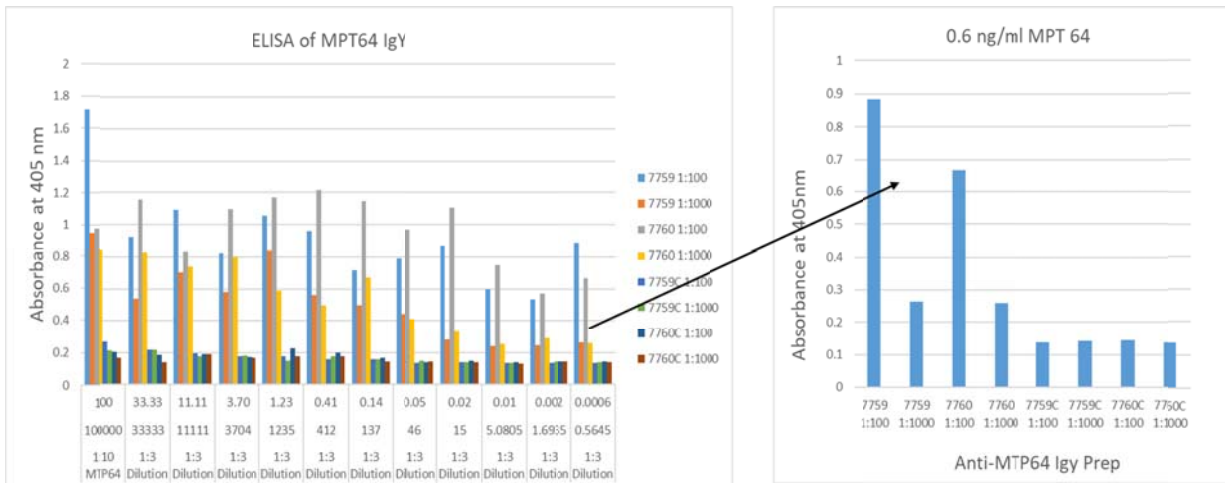


**Figure 5.** (Left). Analysis of the two IgY antibodies generated to the MPT 64 protein. The antibodies, labelled "7759" and "7760" were run at two dilutions (1:100 and 1:1000), and compared to the pre-injection (control) antibodies from the same hen at the same concentration. MPT 64 protein was coated 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 MPT64 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.

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.

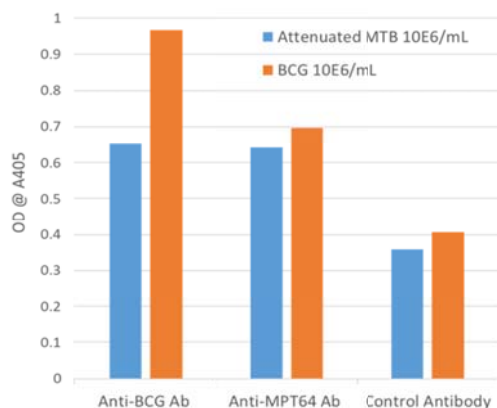


**Figure 6.** Binding of MPT64 antibody and BCG antibody at 28µg/mL to whole *Mycobacterium* cells.

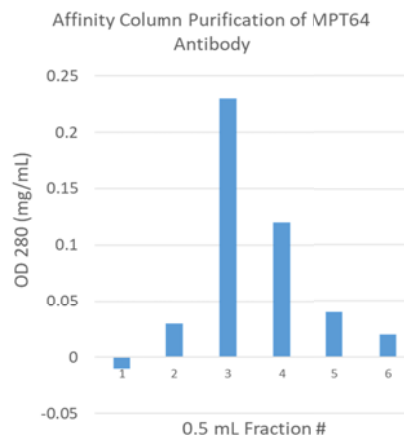


**Figure 7.** Affinity purification of MPT64 antibody using a column of immobilized MPT64.

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.

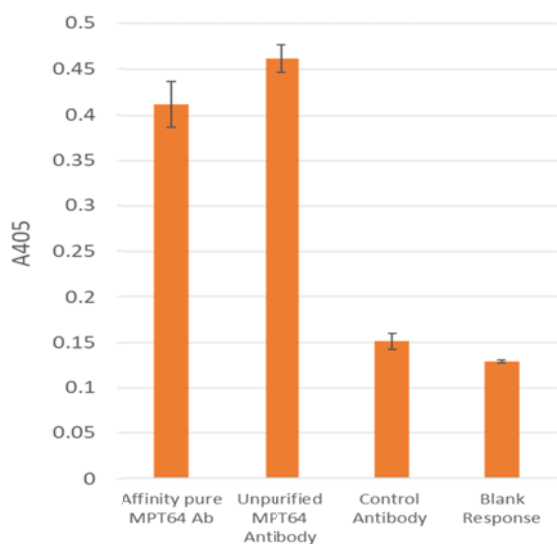


**Figure 8.** ELISA to MPT64

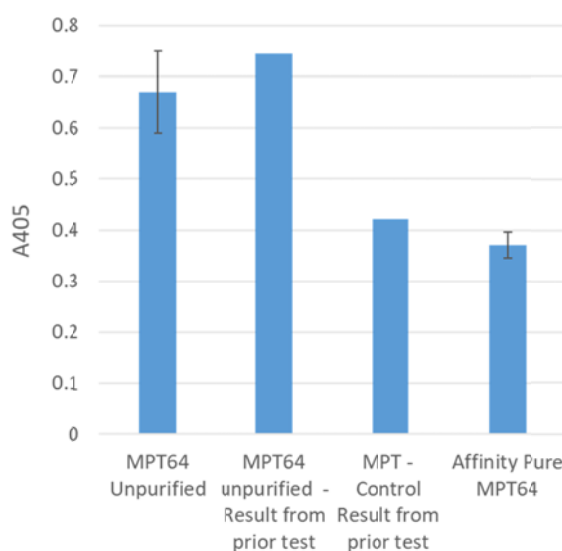


**Figure 9.** Filter EIA to BCG cells

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 MTP64 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  $1 \times 10^7$  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 MTP64 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.

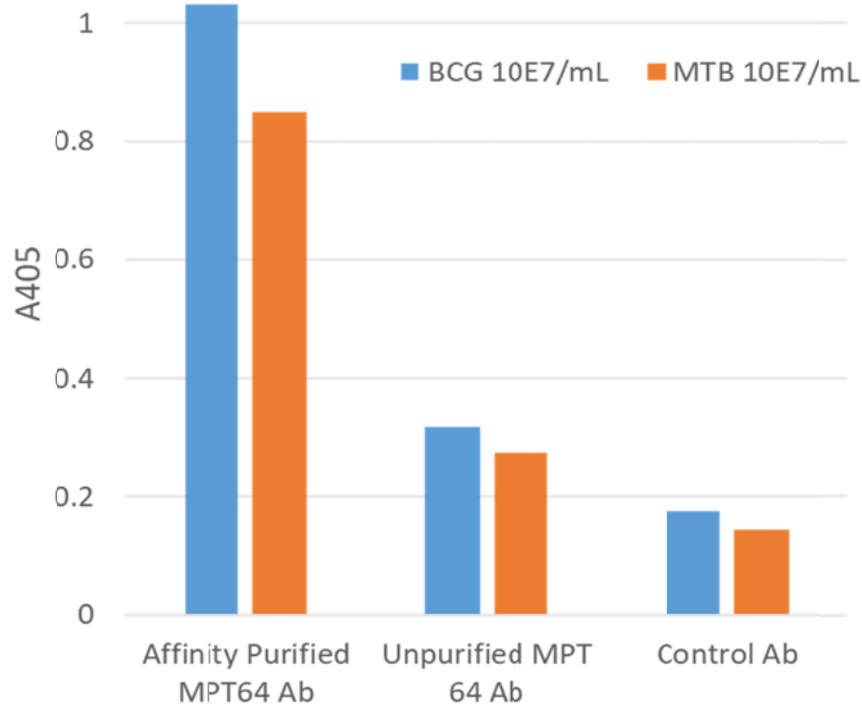


**Figure 10.** ELISA to incubated BCG and MTB H37Ra

## B2. 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. The first step was to measure the baseline signal. One  $\mu$ l 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 $\mu$ l of phosphate buffered saline (1x PBS) was incubated for 10 min and gently removed with a nitrogen gun for 1 min. For the positive control, the same procedure mentioned above was conducted with various concentrations of target-antigen suspended in 1x PBS. After the rinsing step with DI water for 10, the last I-V signal was collected.

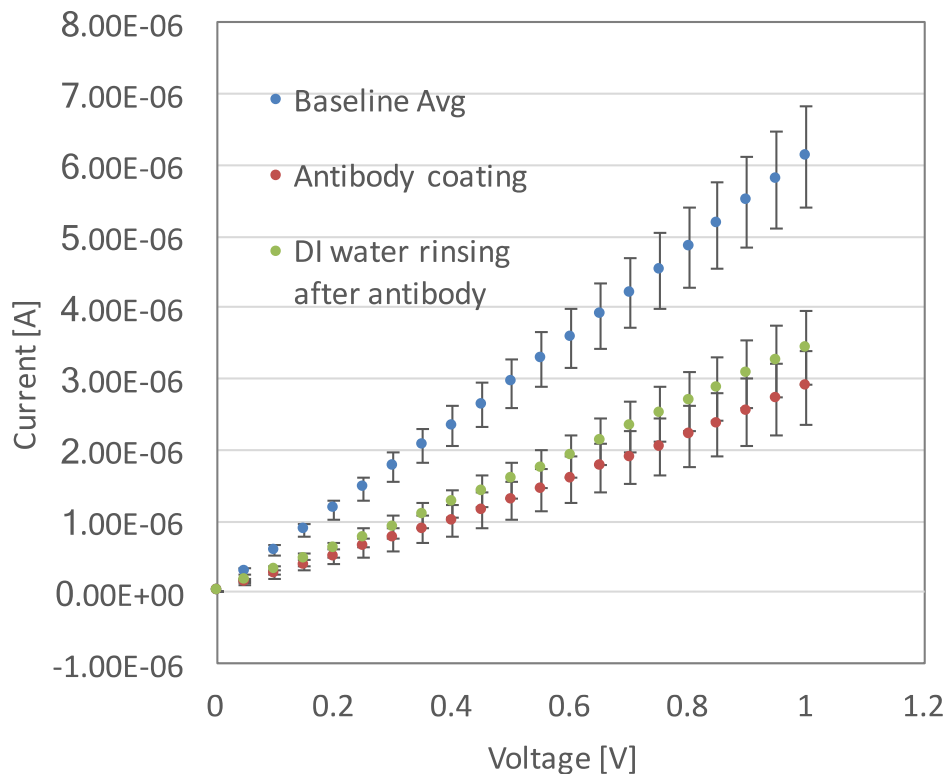


**Figure 11.** Sensor-to-sensor variations for fabricated device (n=8).

In the immobilization step, the binding between antibodies and PEI is ionic binding between negatively charged antibodies and positively charged amine groups. In addition, the 1nm diameter of SWCNT enhances the nonspecific binding. To confirm the function of antibodies, we used fluorescence-labelled BCG cells (10<sup>7</sup>CFU/mL) to determine if the immobilized antibodies react with BCG cells. Fig. 12 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.

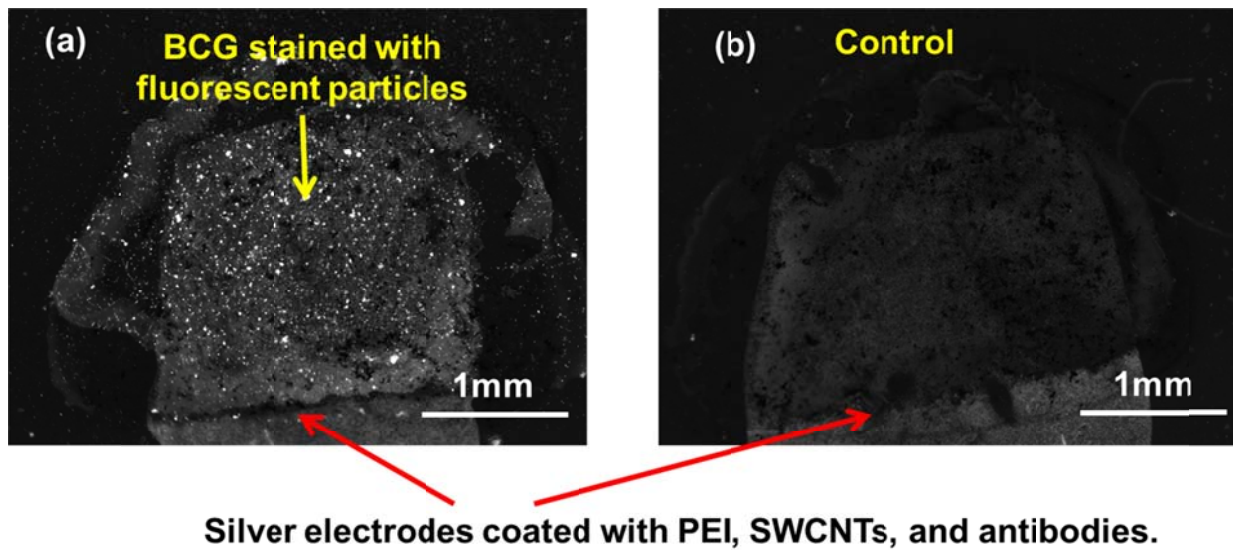


**Figure 12.** Fluorescence images showing the binding between BCG cells and MPT 64 antibodies. (a) Fluorescently stained BCG cells bound on to electrode surface. Electrode consists of silver/PEI/SWCNTs/antibodies. (b) Control electrode without stained cells.

**C. Demonstrate the prototype device with pure samples (Completed dates, April 30, 2018, 60% completion).**

-Preparation of cells and MPT64: Note that all the evaluation results for cells and antibodies are described in the previous section B1.

-Demonstration of sensor performance for BCG in PBS buffer.

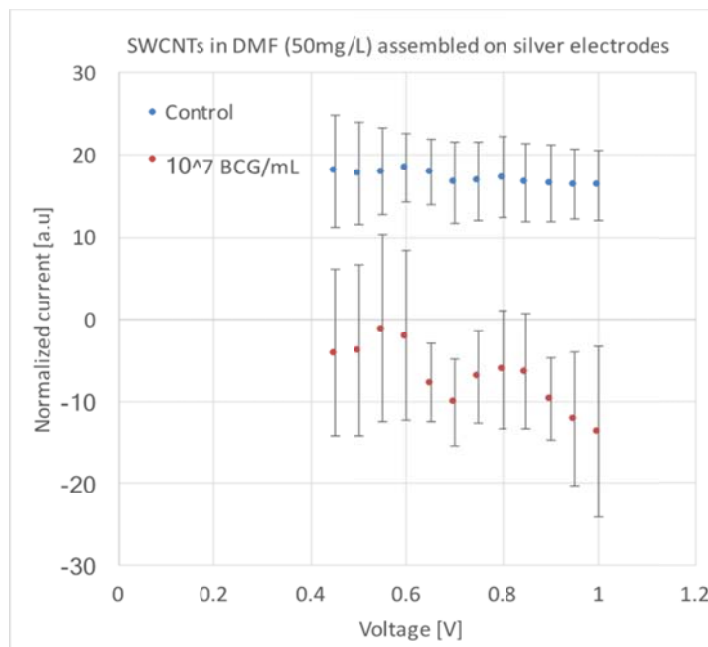


**Figure 13.** Normalized current for the applied voltage between 0 and 1V for negative control and BCG in 1xPBS ( $10^7$  CFU/mL).

The detection of MPT64 in 1x PBS buffer was demonstrated using an optimized protocol. One  $\mu\text{L}$  of affinity purified antibody was applied.

BCG ( $10^7$  CFU/mL) in 1xPBS buffer could be detected by the sensor. Using the various SWCNT sensors, BCG could be detected at the concentration of  $10^6$  CFU/mL. However, the sensitivity rapidly dropped to the control level at the concentration lower than  $10^5$  CFU/mL. The decrease of the signal could be resulted from the screening effect by high concentration ions in PBS buffer. The details of our detection results using the various sensors are described in Table 1. The details of our future plan are described in the section of our future plan.

#### -Demonstration of sensor performance for MPT-64 in PBS buffer.

The detection of MPT64 in 1x PBS buffer was demonstrated using an optimized protocol. One  $\mu\text{L}$  of affinity purified antibody was applied to the device and rinsed with DI-water for 10 seconds to remove excess antibody molecules. We used 10 $\mu\text{L}$  of 1x PBS and various concentrations of MPT64 from 0.1  $\mu\text{g/mL}$  to 10  $\mu\text{g/mL}$  (equivalent to  $8 \times 10^5$  CFU/mL to  $8 \times 10^7$  CFU/mL) for negative and positive controls, respectively. I-V measurement was conducted in between steps and compared.

As shown in Fig. 14a, the detection of MPT64 protein has been demonstrated to evaluate the sensor performance. The developed sensor qualitatively detected the MPT64 protein as low as 0.1  $\mu\text{g/mL}$  in 1x PBS, which is equivalent to  $\sim 8 \times 10^5$  CFU/mL of BCG (Fig. 14 b).

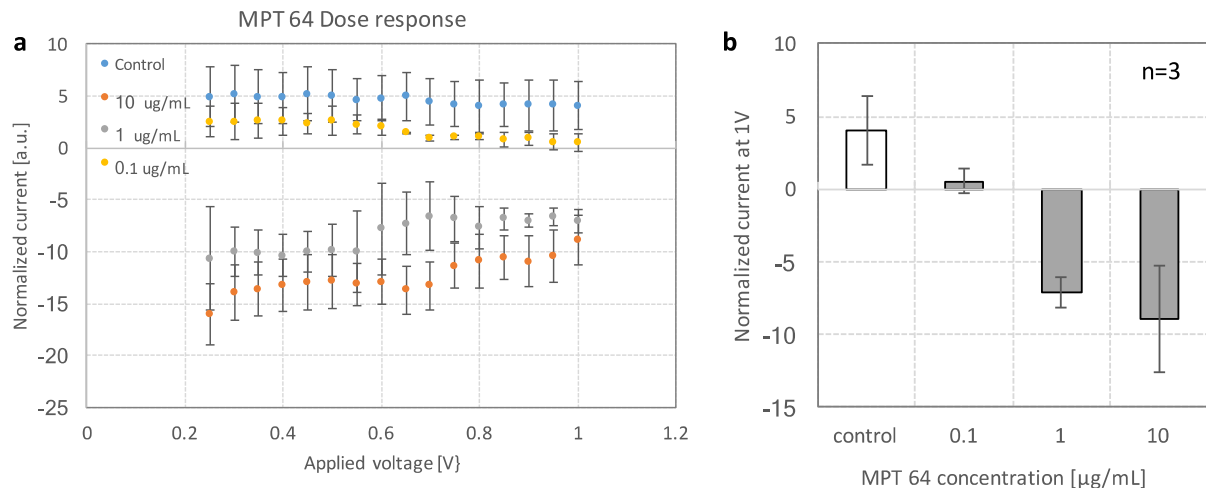
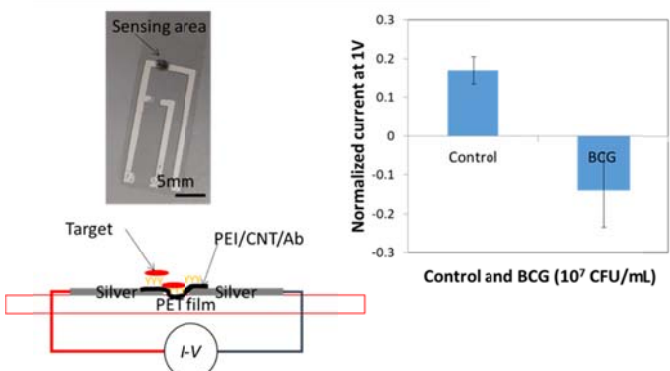
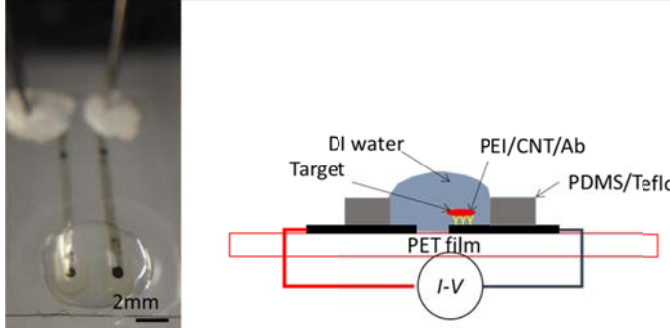
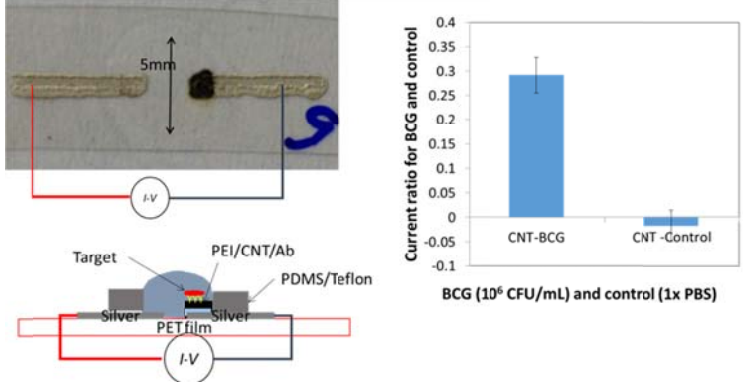
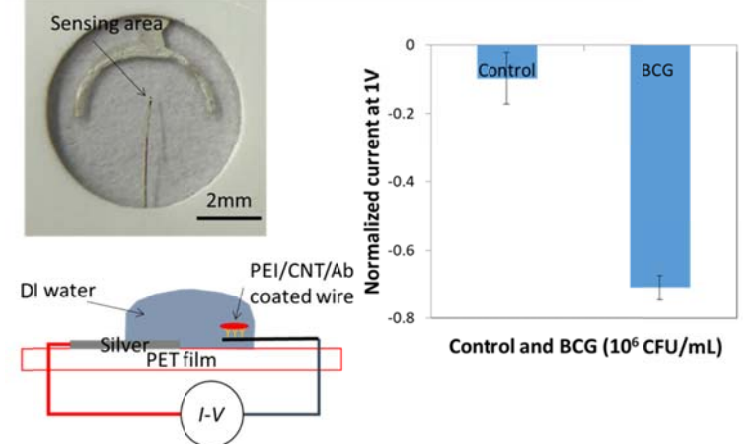


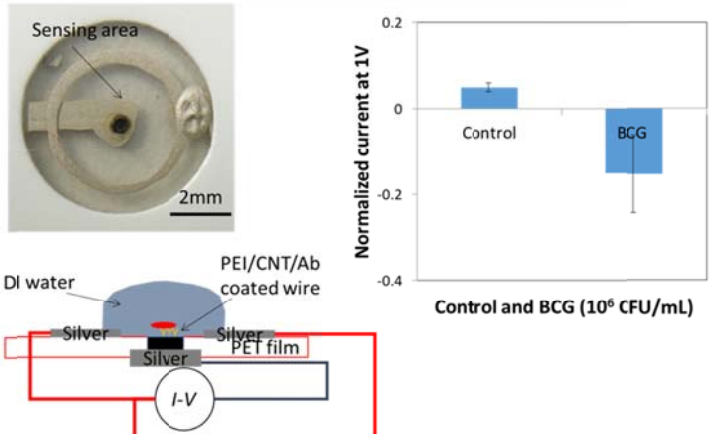
**Figure 14.** Dose response results with MPT64. (a) Normalized current values with applied voltage ranging from 0.25V to 1V for negative control and various concentrations of MPT64 suspended in 1x PBS. (b) Normalized current values at 1V.

In addition to the presented device, 4 more sensors have been fabricated and tested. In consideration of the device performance, reliability and usability, the presented device has been chosen for the further tests. The device

configurations of the presented device are compared with the other devices that have been fabricated with pros and 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<br>(Sept 2017~present)            |  <p>Normalized current at 1V</p> <p>Control and BCG (<math>10^7</math> CFU/mL)</p>                     | Electrostatic gating effect and Schottky effect; measurement without DI water          | Simple fabrication<br>Simple detection protocol               |
| 2. Two prong sensor<br>(May 2017~June 2017)           |  <p>DI water</p> <p>Target</p> <p>PEI/CNT/Ab</p> <p>PDMS/Teflon</p> <p>PET film</p> <p>I-V</p>        | Electrostatic gating effect; Measurement in DI water                                   | Simple fabrication<br>No difference between control and BCG   |
| 3. Two electrode sensor<br>(June 2017 ~December 2017) |  <p>Current ratio for BCG and control</p> <p>BCG (<math>10^6</math> CFU/mL) and control (1x PBS)</p> | Schottky effect and measurement by radial direction of SWCNTs; Measurement in DI water | Simple fabrication<br>Data is not reproducible                |
| 4. Wire sensor<br>(January 2018~March 2018)           |  <p>Normalized current at 1V</p> <p>Control and BCG (<math>10^6</math> CFU/mL)</p>                   | Schottky effect and measurement by radial direction of SWCNTs; Measurement in DI water | Higher sensitivity<br>Fabrication process is not reproducible |

|                                                        |                                                                                   |                                                                                                             |                                                                        |
|--------------------------------------------------------|-----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------|
| 5. Micropore<br>sensor (January<br>2018~March<br>2018) |  | Schottky<br>effect and<br>measurement<br>by radial<br>direction of<br>SWCNTs;<br>Measurement<br>in DI water | Higher<br>sensitivity<br><br>Fabrication<br>process is<br>not reliable |
|--------------------------------------------------------|-----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------|

\*Electrostatic gating effect and Schottky effect: For sensor characterization,  $I(\text{current})$ – $V(\text{potential})$  curves are used as a tool to identify the electronic modulation. Fig. 15 (left) shows the change of the  $I$ - $V$  curve due to an electrostatic gating effect. When charged targets are adsorbed on a SWCNT, the doping effect can shift the  $I$ - $V$  curve along the voltage axis due to partial charge transfer. The  $n$ -doping of the SWCNT shifts the  $I$ - $V$  curve to more negative gate voltages. In case of the Schottky barrier (Fig. 15, right), adsorbed targets on a SWCNT modulate the local work function and thus the band alignment. Because the Schottky barrier height changes in opposite directions for hole (p) and electron (n) transport (see insets), an asymmetric conductance change for p- and n-branches of  $I$ - $V$  is observed. Through the various configurations, 5 kinds of sensing platforms were tested. The sensor platform bridging two silver electrodes by SWCNTs was turned out to show a reliable signal. In addition, the fabrication of the chosen sensor was one of the simplest formats with performance for multiplexing.

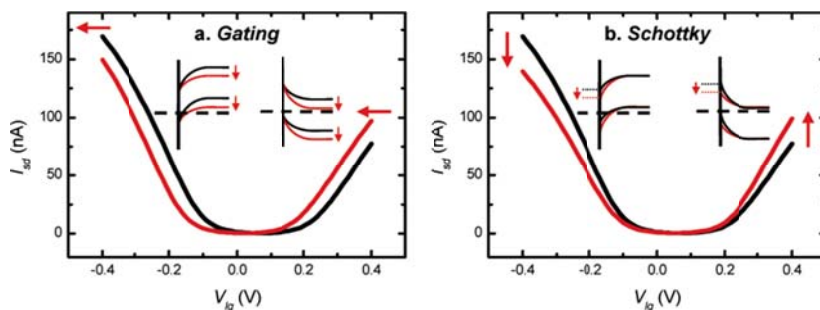


**Fig. 15** Computed  $I$ - $V_{lg}$  curves before (black) and after (red) protein adsorption for *gating* and *Schottky* barrier effects (Reference: Janssens, HI et al, Nano Lett. 2008;8(2):591-5)

**In conclusion**, the fabrication process for the sensor was accomplished with optimization of the fabrication protocols. For specific binding, antibodies to MPT64 were generated from hens. The polyclonal antibodies showed binding affinity to MPT64, MTB, and BCG. Through affinity purification, the binding affinity to MPT64 and MTB was significantly enhanced. Four kinds of sensors were fabricated and tested for detection of BCG and MPT64. Among them, a sensor composed of SWCNTs bridging silver electrodes was chosen to test a dose response to MPT64 and BCG. The major challenge of the electrical detection was the screening effect of high-concentration ions on SWCNT sensor surface. As the ion concentration increased with PBS buffer, the screening

effect by ions significantly reduced the sensitivity of the sensor. The protocol optimization step required significantly more time than initially planned. Through the development of a washing step using deionized water, the excessive ions from PBS buffer could be removed to increase the sensor sensitivity. However, the doping effect by PBS was too dominant to achieve the detection limit of  $10^3$  CFU/mL. Our plan in the next period will be described in the plan section.

**In summary, the followings are the major accomplishment of the project according to the proposed plan,**

**A. Design and fabrication of SWCNTs-based immunosensors (Completed dates: March 15, 2018; 100% completed).**

- Fabrication and optimization of SWCNT based sensors and fabrication parameters

**B. Sensor optimization for specificity and sensitivity using pure samples (Completed dates: March 15, 2018; 100% completed).**

- Generation and characterization of antibodies to MPT64
- Affinity purification of MPT64 antibodies for higher sensitivity to MTB and MPT64
- Optimization of functionalization steps for a SWCNT immunosensor.

**C. Demonstrate the prototype device with pure samples (Completed dates, April 30, 2018, 60% completed).**

- Dose response test for MPT-64 detection (100 ng/mL corresponding to  $8 \times 10^5$  CFU/mL)
- Initial test for BCG detection ( $10^6$  CFU/mL)

▪ **What opportunities for training and professional development has the project provided?**

Graduate student training: The PI works closely with a graduate student on the project. The experimental and multidisciplinary nature of this project allows the student to learn and gain various hands-on experiences and develop extensive laboratory skills.

At University of Washington, a graduate student, Seong-Joong Kahng was trained by the project. He fabricated and ran the bioassay.

At Washington State University, a graduate student, Fabrice Fondjo, was trained by the project. He fabricated the device and performed the experiment.

**How were the results disseminated to communities of interest?**

A manuscript about the noncontact printing method of SWCNTs was accepted to Nanotechnology, which will be published soon. The accepted manuscript is attached in the appendix. At University of Washington, Engineering discovery days were held to expose K-12 students to new engineering technology. Chung's group hosted K-12 students in Engineering Discovery Days (April 19 and 20). The sensor prototype was demonstrated to K-12 students (Fig. 16a).

Kim's group at Washington State University presented the research outcomes in this project at the Research Showcase on April 17, 2017 (Fig. 16b).

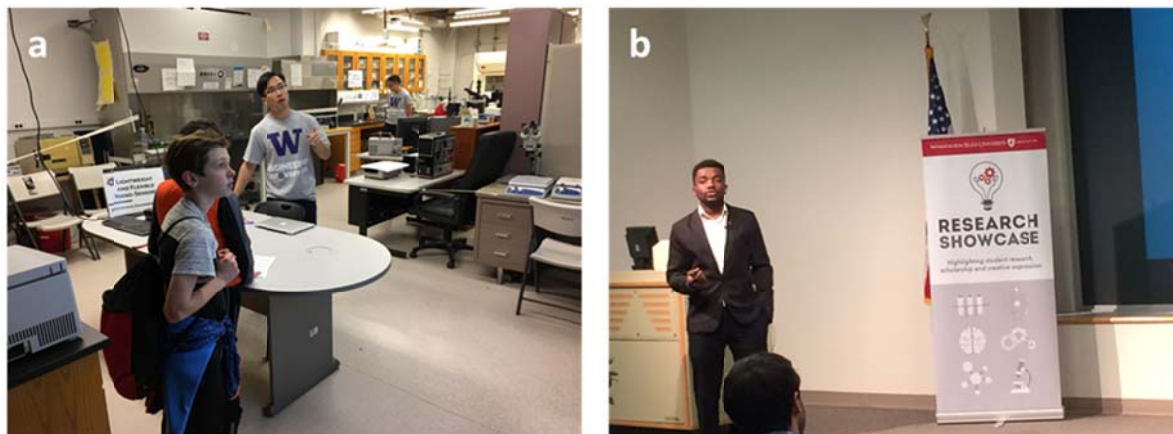


**Fig. 16.** (a) Sensor demonstration to K-12 students on Engineering Discovery days at University of Washington. (b) Research showcase presentation to faculty, staff, and students at Washington State University

#### **What do you plan to do during the next reporting period to accomplish the goals?**

In the next project period, the detection protocol will be further optimized to enhance the sensitivity to  $10^3$  CFU/mL. Specifically, the gap size between silver electrodes will be reduced to  $100\mu\text{m}$  to use the Schottky effect and reduce the electrostatic gating effect. If the sensitivity is enhanced, the dose response test for BCG (MTB) will be conducted to complete the milestone. Subsequently, the test using sputum spiked with BCG (and MTB) will be conducted to complete the remaining project.

If the sensitivity is not enhanced by the optimization, we will use magnetic beads to concentrate target cells (MTB) and MPT-64. The enriched targets will be suspended in a low concentration buffer (for example,  $0.1 \times \text{PBS}$ ), which will increase the sensitivity. Co-PI, Dr. Furlong is an expert using magnetic beads for concentration and purification of target bacteria, protein and nucleic acids from human samples. Once developed, it will be able to simultaneously detect multiple targets with a high sensitivity.

For this project, we requested no-cost extension to extend the project period for one more year. We hope to get approval.

4. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

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

This innovative approach based on nanoink printed amperometric immunosensor will be critical in point of care diagnosis of tuberculosis (TB). The proposed work will establish a reproducible nanoink printing method for cost-effective manufacturing of the amperometric immunosensor. The immunosensor will be capable of detecting MTB and protein biomarkers (MPT64) in sputum samples with detection limit as low as 1,000 CFU/mL in 20 minutes.

▪ **What was the impact on other disciplines?**

The printing technology of SWCNTs will have impact on large scale fabrication of physical, chemical and biosensors. In addition, the fabrication steps will offer a stepping stone for scalable nanomanufacturing. See the attached manuscript in the appendix.

▪ **What was the impact on technology transfer?**

Nothing to report

▪ **What was the impact on society beyond science and technology?**

In this project, we are developing a diagnostic assay that can be used at the point of care to rapidly and accurately diagnose TB. With nanoink printed amperometric immunosensor, we aim to achieve a detection limit of 1,000 CFU/mL with the detection time of 20 minutes in sputum samples, which will be faster and at significantly lower cost than skin tests, smear microscopy and PCR. 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 positive, which will spare the patient unnecessary toxicity of a drug that does not provide benefit. Therefore, the immunosensor can offer an immediate solution that can address the current challenge of rapid, inexpensive and accurate TB diagnosis in low resource settings.

5. **CHANGES/PROBLEMS:**

There is no significant change in the proposed plan. To successfully accomplish the proposed plan, we requested no cost extension of the project. The requested new end date is October 30, 2019, which is one more year from the original termination date. Due to the short project time period (18 months), additional time beyond the original end date is required to ensure adequate completion of the originally approved project. In addition, the extension is necessary to allow an orderly phase-out of a project that will not receive continued support. In other words, we would like to finish the project with more complete outcomes, which will potentially lead the developed platform ready for future funding and clinical tests.

- **Changes in approach and reasons for change**

Nothing to report

- **Actual or anticipated problems or delays and actions or plans to resolve them**

As mentioned in the conclusion, the screening effect of high concentration ions in PBS significantly lowered the sensitivity of the SWCNT sensor, which was resolved by introducing deionized water for washing before electric measurement. We will optimize the detection protocol further in order to obtain a reliable protocol.

- **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**

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

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

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**

*A manuscript about carbon nanotube printing was accepted to Nanotechnology, which was. See the attached manuscript in the appendix.*

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

|                                        |                                                                                                            |
|----------------------------------------|------------------------------------------------------------------------------------------------------------|
| Name:                                  | <i>Jae-Hyun Chung</i>                                                                                      |
| Project Role:                          | <i>PI</i>                                                                                                  |
| Researcher Identifier (e.g. ORCID ID): | <a href="https://orcid.org/0000-0002-9861-8559">https://orcid.org/0000-0002-9861-8559</a>                  |
| 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): | <a href="https://orcid.org/0000-0001-8010-7753">https://orcid.org/0000-0001-8010-7753</a>                  |
| 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): | <a href="https://orcid.org/0000-0002-6489-7211">https://orcid.org/0000-0002-6489-7211</a>                  |
| 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>Fabrice Fondjo</i>                                                                                                     |
| Project Role:                          | <i>Graduate Student</i>                                                                                                   |
| Researcher Identifier (e.g. ORCID ID): | N/A                                                                                                                       |
| Nearest person month worked:           | 9                                                                                                                         |
| Contribution to Project:               | <i>Mr. Fondjo has conducted experiments and collected data.</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?**

Nothing to report

#### 8. **SPECIAL REPORTING REQUIREMENTS**

Nothing to report

9. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc. Reminder: Pages shall be consecutively numbered throughout the report. **DO NOT RENUMBER PAGES IN THE APPENDICES.***

# **Nanoink Bridge-induced Capillary Pen Printing for Chemical Sensors**

**Seong-Joong Kahng<sup>1</sup>, Chiew Cerwyn<sup>1</sup>, Brian M. Dincau<sup>2</sup>, Jong-Hoon Kim<sup>2</sup>, Igor V. Novosselov<sup>1</sup>, M. P. Anantram<sup>3</sup>, and Jae-Hyun Chung<sup>1</sup>**

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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 (PET) 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.

## 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, 2, 4]. 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[12, 21, 25] rely on thermal expansion or electromechanical vibration to eject droplets[21, 23]. Due to the droplet formation physics, inkjet printing is challenging for nanoink of low viscosity and high surface tension[6, 13] which often requires significant modifications of ink properties[24, 26, 27]. 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[5, 13, 17]. Electrohydrodynamic printing utilizes an electric field to control flow through a nozzle[11, 18, 19], but it is limited to a conductive and semi-conductive substrate. Stencil printing[15] deposits nanomaterial by spraying through a mask, which is limited to the designed masking pattern.

As an alternative, fountain pens[3, 9, 22], ball pens[7], and pencils[14, 16] 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[10]. However, printing on an impermeable film is more challenging as the contact angle is much greater than  $0^\circ$ . Moreover, any contact printing may damage the substrate[9] hindering multiple layer deposition required for complex sensor structures.

In this paper, a noncontact 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 noncontact capillary method deposits nanoink through a liquid bridge forming between a capillary pen and substrate (Figure 1a). 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 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,  $\theta_{B\_a}$  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 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 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 1c 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  $H=100\text{ }\mu\text{m}$ ; 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 1d 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.

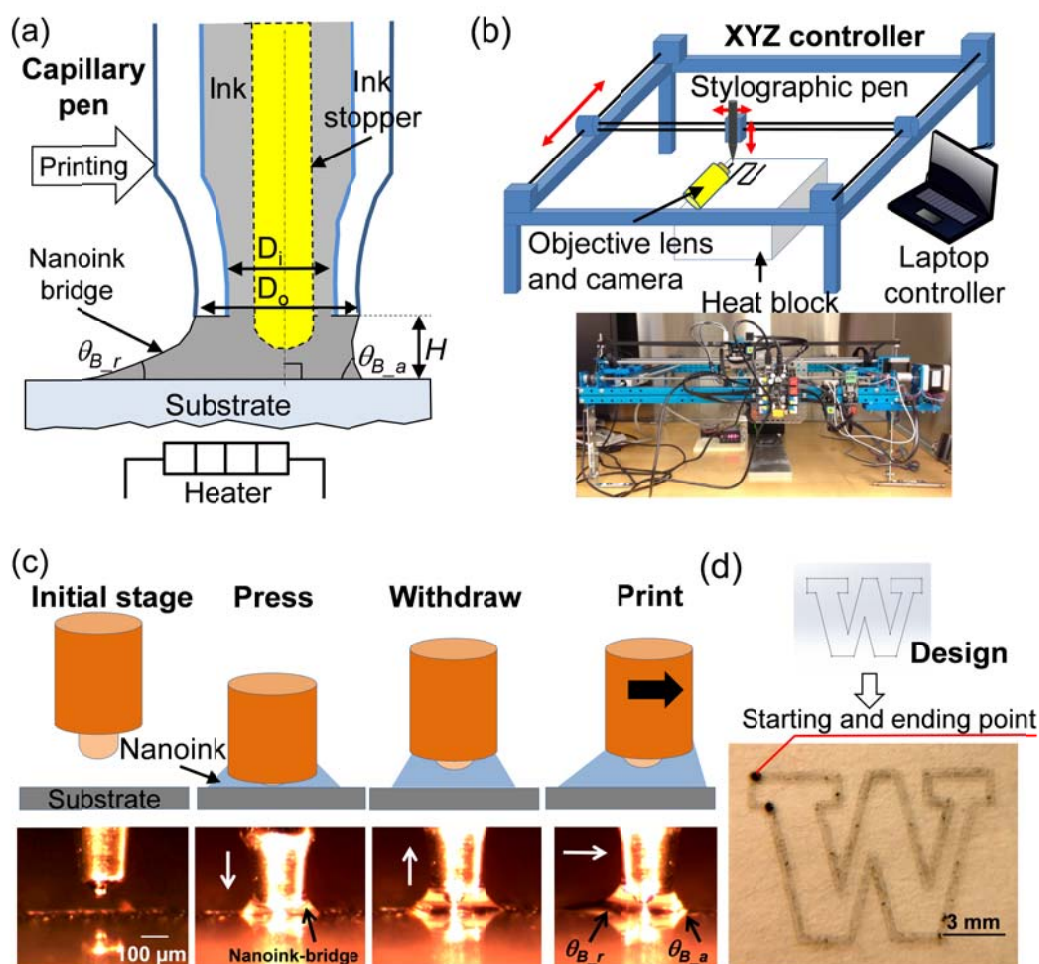


**Figure 1.** Nanoink bridge induced capillary printing (a) Printing concept (b) Schematic of an xyz plotter installed with a heating stage and a camera system. The top image shows a printing system, and the bottom is a photograph of the setup. (c) Nanoink-bridge induced printing using water ink on a PET film. The ink is released with 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°C at 1.2 mm/sec. The top image shows a design.

### 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 (5mg/mL) in 1% sodium dodecyl sulfate (SDS) by sonication. In the suspension, 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 1a) were 225, 375, and 790  $\mu\text{m}$ , respectively (Supplementary information; Figure S1). 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 chamber. The temperature and relative humidity in the chamber were  $26 \pm 1.0$  °C and  $35 \pm 2.0$  %, respectively.

**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 $\mu\text{m}$ -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 seconds at each temperature. The contact angles ( $\theta_b$ ) 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).

**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 mm/s, and the

temperature was controlled from 20 to 100 °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 (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 mm<sup>2</sup>. Printing speed was set for 2.5 mm/sec, and the substrate temperature was varied from 20 to 100°C. The nominal diameter of a capillary pen was 700 μm. To achieve complete coverage, the pen was shifted by 600μm 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).

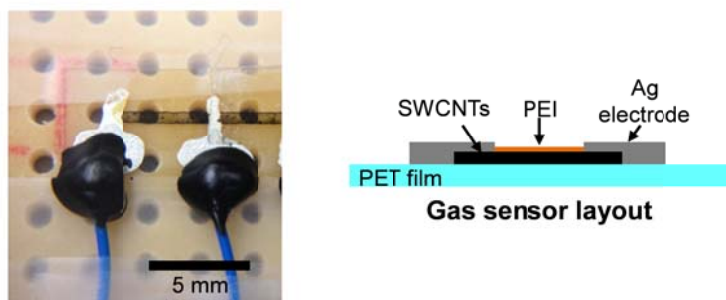
### 3.2 Doping effect

The electrical characteristics of 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 (1μL) 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.

### 3.3 Sensor fabrication

**Gas sensor** To fabricate a gas sensor, SWCNT electrode was printed as a line using a 300 $\mu$ m-diameter pen (Figure 2a). 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  $\mu$ 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  $\mu$ 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 NO<sub>2</sub> (8 and 22 ppm). The sensors' resistance was measured and recorded by a multimeter (287 True RMS Multimeter, Fluke Corporation).

(a)



(b)

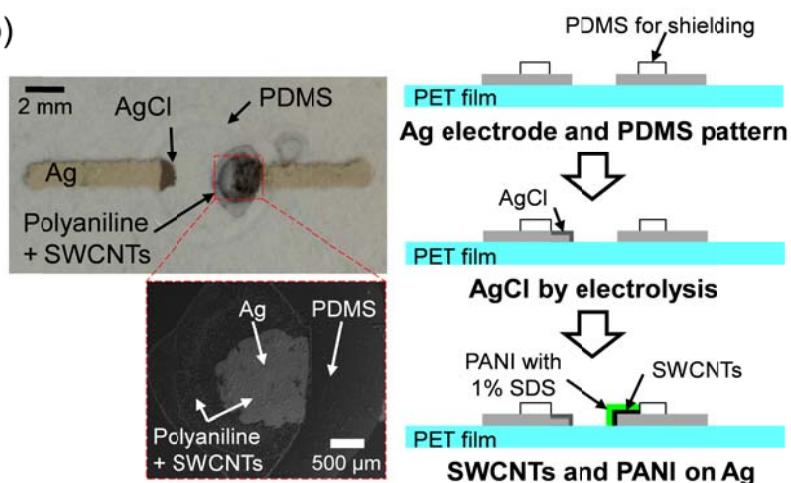


**Figure 2.** Fabrication steps (a) Images and cross-section of a SWCNT-gas sensor (b) Optical and SEM images and fabrication steps of a SWCNT-pH sensor.

**pH sensor** Silver electrodes were screen-printed on PET film (Figure 2b), 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 hour in a convection oven. One of the silver electrodes (cathode) was modified to form an AgCl layer by electrolysis (1.5Vdc for 1 minute in 1M HCl solution). For the other electrode (anode), SWCNT lines were printed on silver electrodes with speed of 0.83 mm/sec using a 300  $\mu$ m-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 mm<sup>2</sup>. The printing conditions were 80 °C. 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  $\mu$ L 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 0~3V, a 1.61V AA battery was serially connected to shift the voltage potential above 0V in the control pH ranges (pH 7~10).

## 4. Results and discussion

### 4.1 Characterization of printing method

**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 patterns formed when a capillary pen was pressed and retracted to 100  $\mu$ m on a PET film for 1, 5, and 10 seconds (Figure 3a). As the temperature increased, the ratio of the dot

diameter to the outer nib diameter decreased and approached unity (Figure 3b). The dot size reduction was attributed to the increase of the static contact angle ( $\theta_{B_a}$ ) 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  $\theta_{B_a}$  (Figure 3c). As  $\theta_{B_a}$  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 seconds as the temperature increased from 20 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 3d). 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** The section aims to characterize printed lines at various temperatures and printing speeds. In the results, 60 and 80°C showed uniform line width under the printing speed of 2.5 mm/sec 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 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  $\theta_{B_a}$ .

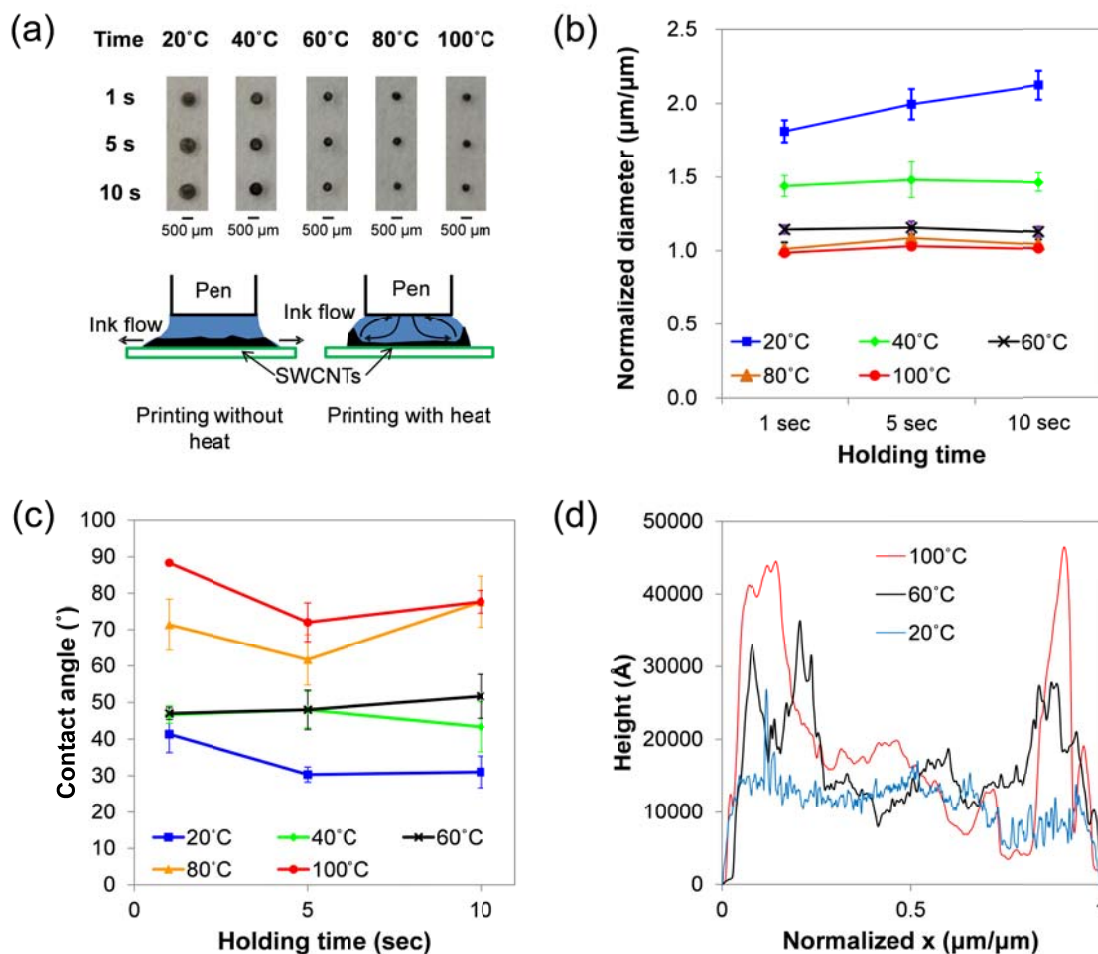


**Figure 3.** Dot printing (a) Dots printed at surface temperature 20~100 °C with holding time of 1, 5, and 10 s. Scale bar: 500  $\mu\text{m}$  (b) Dot diameters normalized by an outer nib diameter (c) Contact angles according to holding time (d) Dot profile at 20, 60, and 100 °C (1s holding time).

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 4a and 4b). 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 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  $\theta_{B_a}$  (Figure 4c) 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, as  $\theta_{B_a}$  approaches  $90^\circ$ .

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  $90^\circ$  for the faster prints. The high  $\theta_{B_a}$  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  $T=100^\circ\text{C}$  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 wider print line. At the higher speeds ( $>2.5$  mm/s), the normalized line width reduced, which was consistent with the increase of  $\theta_{B_a}$  as shown in Figure 4c.

Related to the optimization of the print conditions: at the temperatures greater than  $60^\circ\text{C}$ , SWCNTs could be printed as  $\theta_{B_a} > 90^\circ$ . At the temperature of 80 and  $100^\circ\text{C}$ , a stick-slip effect was observed at the advancing meniscus of a nanoink bridge. The beach mark pattern in Figure 4a 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  $> 60^\circ\text{C}$  while a flat profile was observed at the temperature  $< 60^\circ\text{C}$ . The thickness of the printed line was well below  $1\mu\text{m}$ , 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 (Supplementary information; Figure S4).

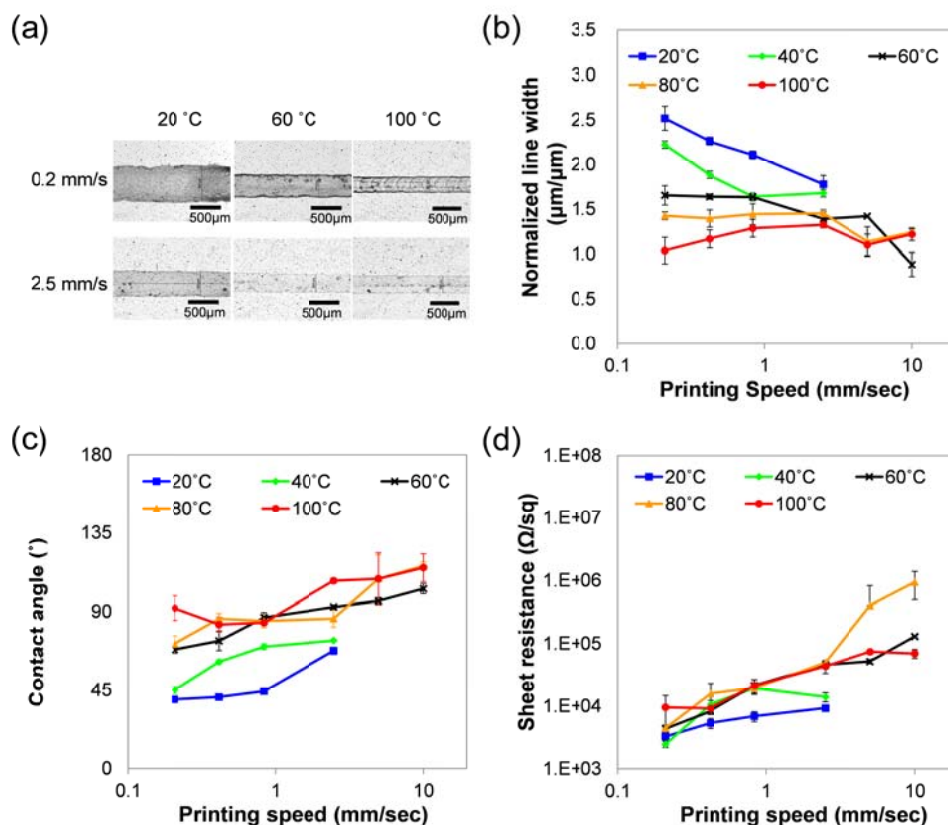


**Figure 4.** Line printing (a) Printed lines at 0.2 and 2.5 mm/s under the substrate temperature of 20~100 °C. (b) Normalized line widths at a temperature of 20~100 °C with a printing speed of 0.2~10mm. (c) Advancing contact angle ( $\theta_{B,a}$ ) at various printing speed. (d) Sheet resistance according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

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 4d). 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 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.

**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 15x15 mm<sup>2</sup> was printed in 3 minutes with the nib speed of 2.5 mm/sec (supplementary 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 5a 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 5b), 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.

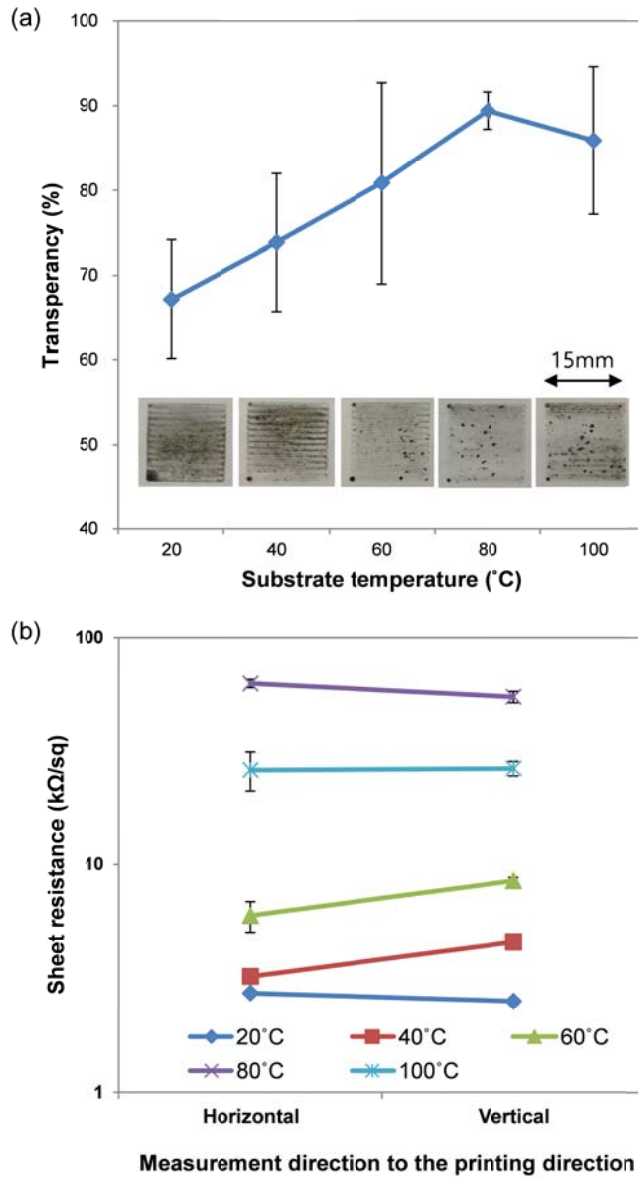


**Figure 5.** (a) Transparency of printed films at temperatures of 20~100°C. Printing speed: 1 mm/s (b) Sheet resistance in the printing and its vertical directions.

#### 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 6a) based on *I-V* characterization (inset figure of Figure 6a). For the repeated tests

(N=3), the error bars for the electric current showed  $\pm 9.5$ ,  $\pm 6.8$ ,  $\pm 5.3$ ,  $\pm 1.6\%$  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 6b).

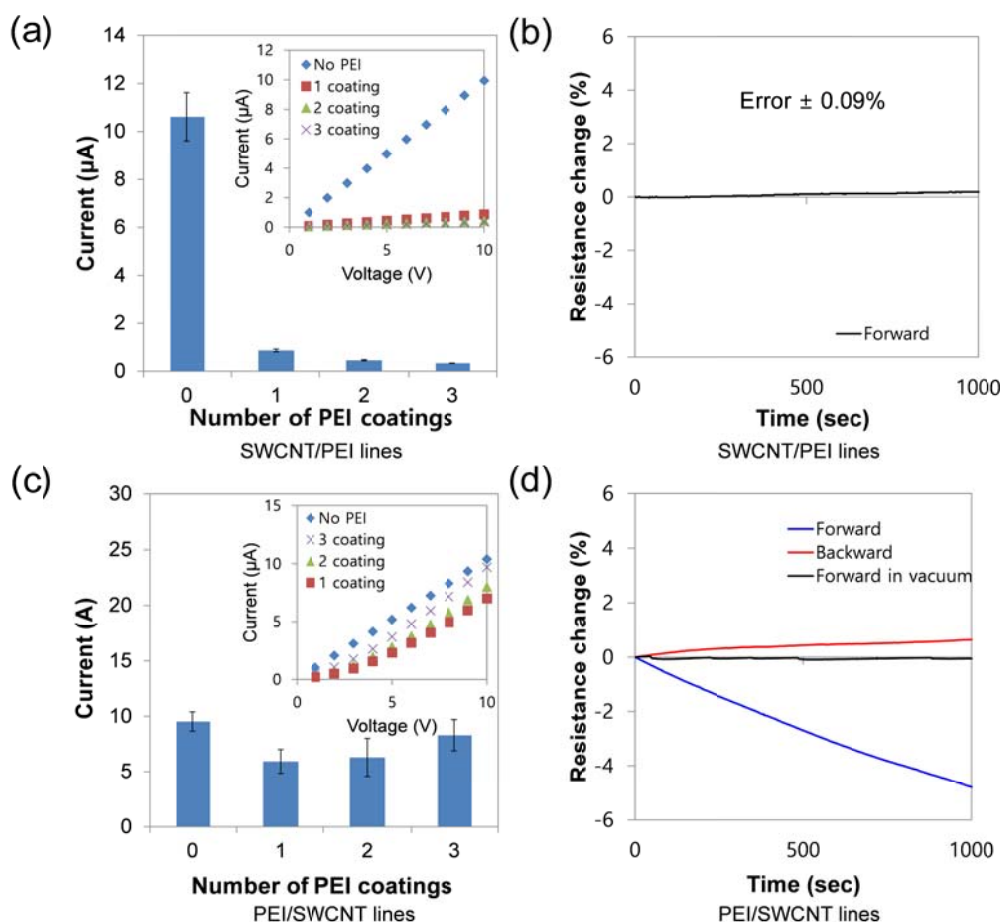


**Figure 6.** Resistance change for SWCNT/PEI and PEI/SWCNT lines (a) Current change at 10 V for 1, 2, and 3-PEI depositions. (b) Resistance change for a SWCNT/PEI line. (c) *I-V* characteristics of PEI/SWCNT lines for 1, 2, and 3-PEI depositions. (d) The resistance change of a SWCNT device for forward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

For PEI/SWCNT lines, the doping effect was not as reproducible as the SWCNT/PEI lines (Figure 6c); the SWCNTs might not be fully covered with PEI. The resistance could increase or decrease for forward and backward printing directions (Figure 6d). 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 low oxygen environment (Figure 6d). Figure 6c and 6d 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

**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 NO<sub>2</sub> while nafion doped SWCNTs could selectively detect NH<sub>3</sub>.

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 NO<sub>2</sub> gas (Figure 7a). Nafion-doped SWCNTs showed a negligible change when exposed to NO<sub>2</sub> gas but had a significant response to ammonia (Supplementary information; Figure S9). The specificity of the SWCNT-sensors to NO<sub>2</sub> and ammonia gases were consistent with the previous report[20]. 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[28]. 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 SWCNTs printed on a PET film while the previous report used semiconducting SWCNTs grown on the micromachined electrodes.

**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[8]. In comparison to the previous report, the fabrication method presented in this paper is more cost-effective.

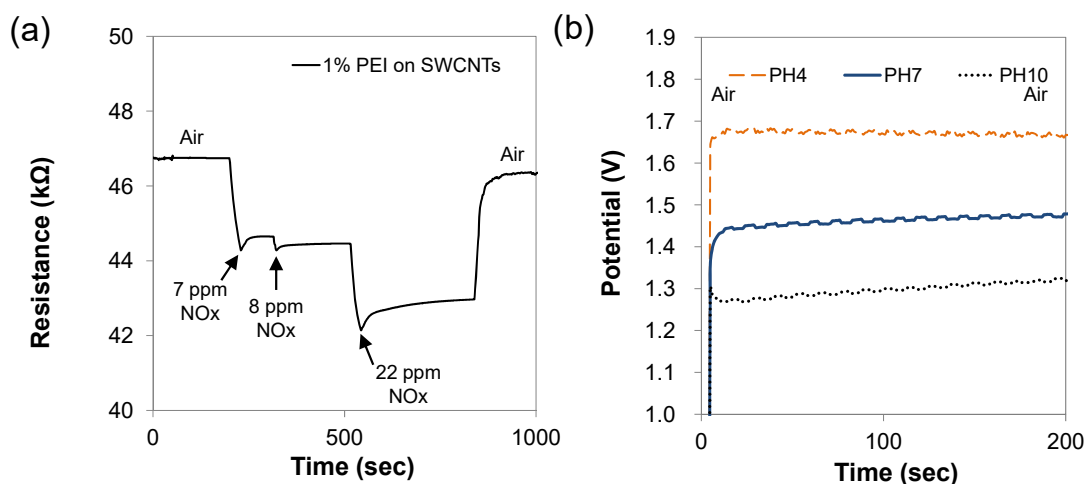


**Figure 7.** Gas and pH response test (a) Change of PEI-doped SWCNT resistance for NO<sub>2</sub> gas; **PEI is doped on a SWCNT line.** (b) Voltage measured for a pH sensor using standard solutions of pH 4, 7, and 10. Note that the voltage is shifted by using a 1.61 V-AA battery.

#### 4.4 Discussion

Due to 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  $\mu\text{m}$ ) and water surface tension coefficient (0.073 N.m), the pressure difference ( $\Delta P$ ) at the substrate surface could range from 0 to 1.3 kPa. At room temperature,  $\Delta P$  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 in the system, the noncontact nanoink-bridge induced printing method can be beneficial for printing water-based molecular ink.

## 5. Conclusions

In summary, we developed a noncontact 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 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, an optimal printing temperatures are in the 60~80  $^\circ\text{C}$  range. A film could be printed to obtain an average sheet

resistance of 7.2 k $\Omega$ /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.

### Acknowledgements

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## **Supplementary Information**

### **Nanoink Bridge-induced Capillary Pen Printing for Chemical and Biological Sensors**

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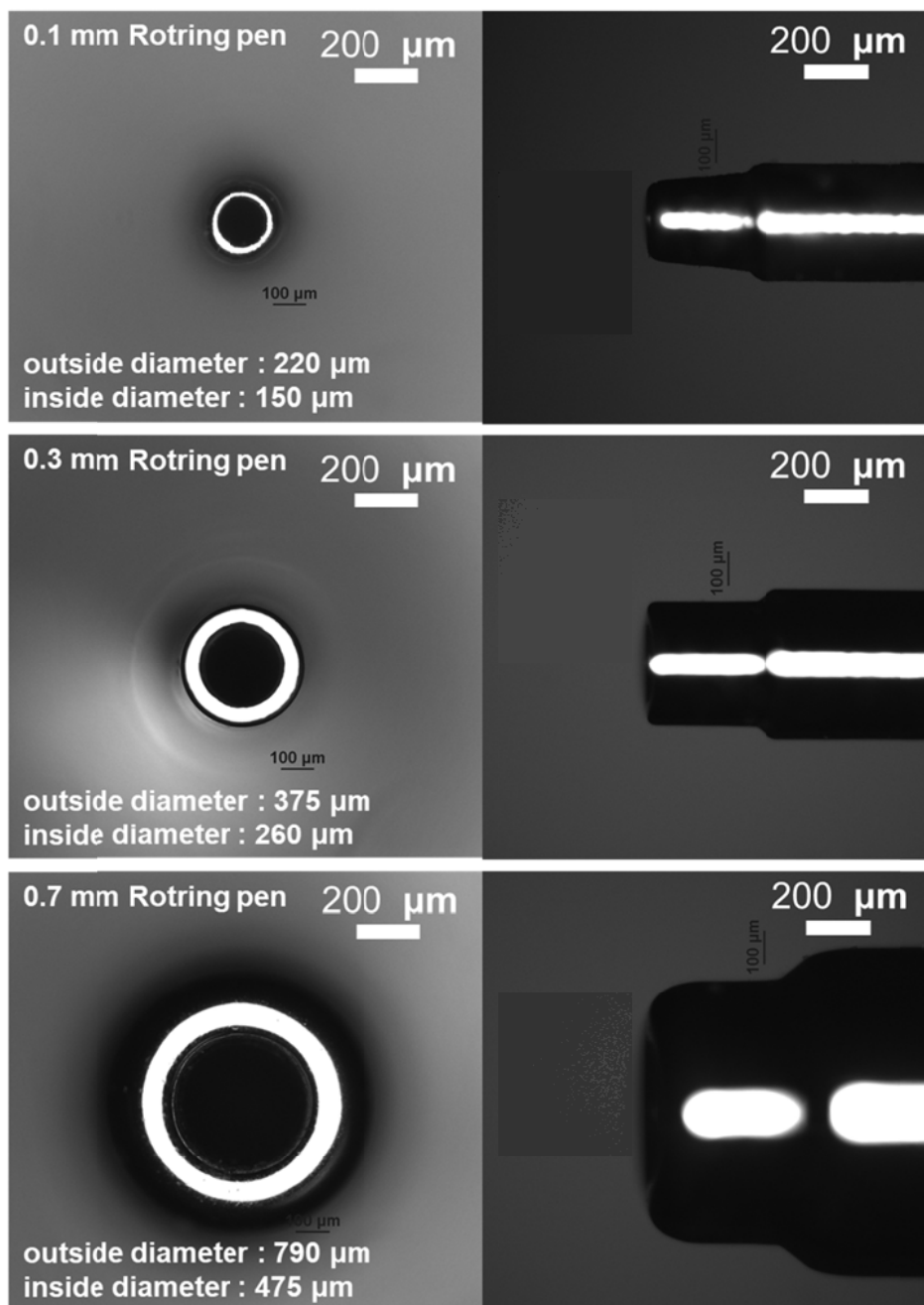


**Figure S1.** Diameters of the capillary pens. The nominal diameters of the capillary pens are 100, 300, and 700  $\mu\text{m}$ . The outer diameters of the pen nib are 225, 375, and 790  $\mu\text{m}$ , respectively.

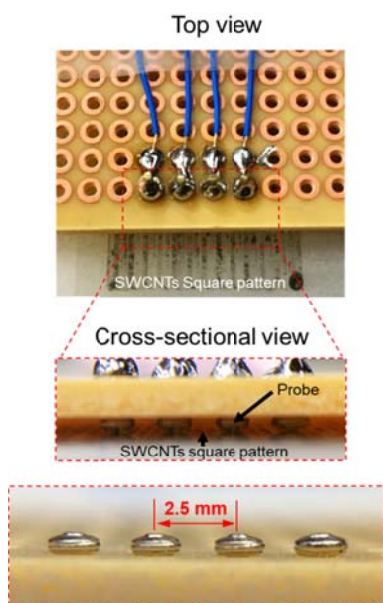


**Figure S2.** 4-point probe measurement setup. The sheet resistance is measured using a custom 4-point probe measurement system. The distance between electrodes is 2.5 mm.

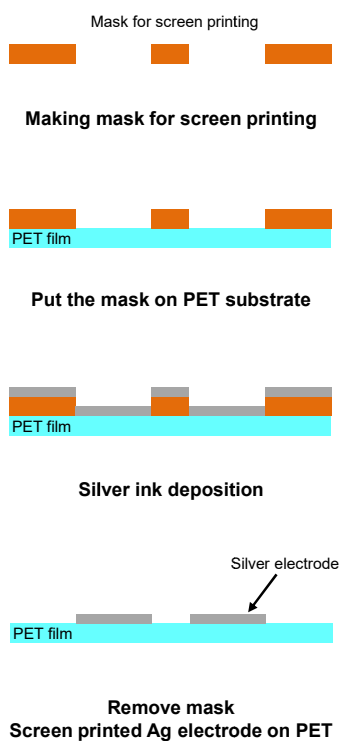


**Figure S3.** Silver electrode patterning. Sensor electrodes for a pH sensor are screen-printed to form silver electrodes on a PET film. The mask material for screen printing is PET film

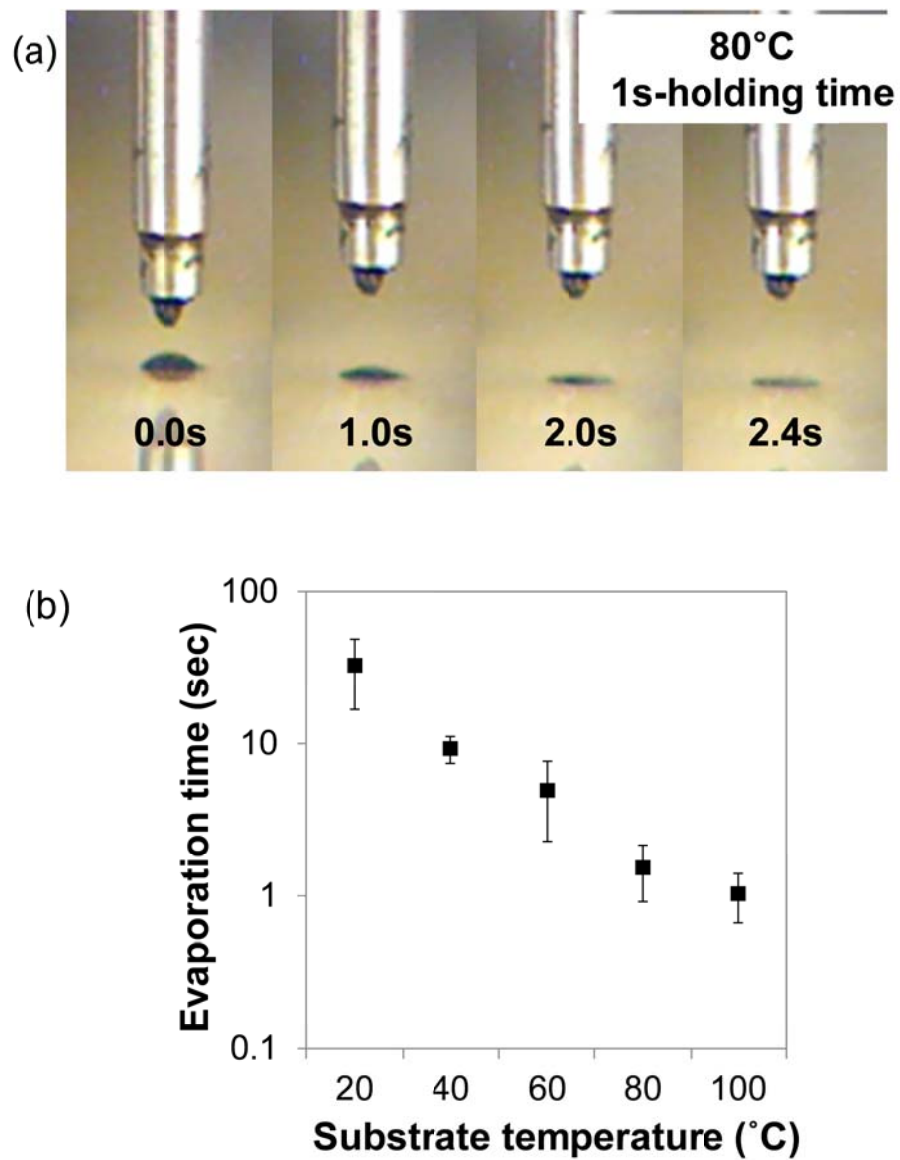


**Figure S4.** Evaporation of a dot with 1s-holding time at temperatures from 20°C to 100°C. (a) Evaporation of nanoink after 1s of holding time for dop printing at 80°C. (b) Evaporation time according to substrate temperature.

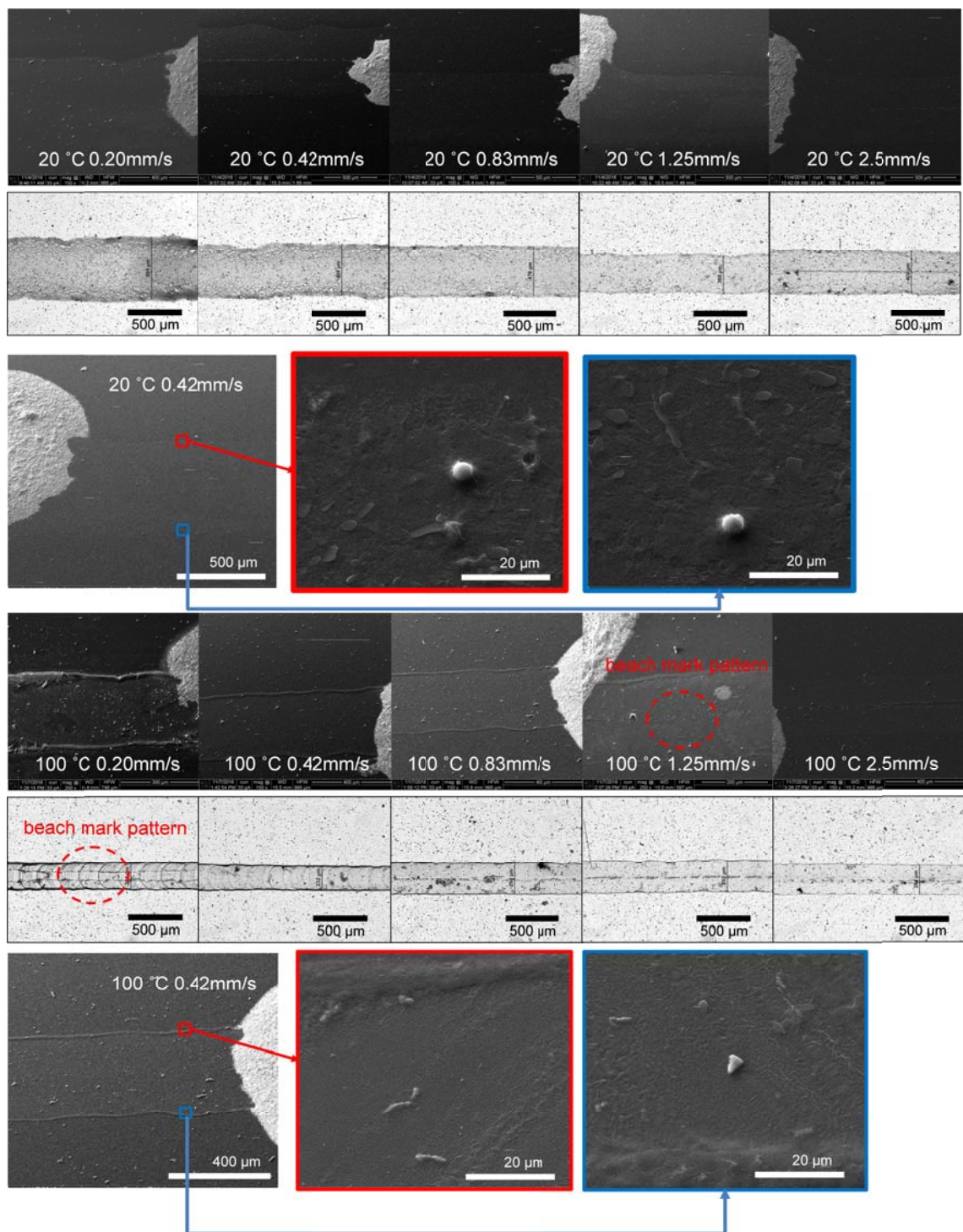


**Figure S5.** Printed SWCNT lines at 20 and 100 °C. For each set of images, the first rows are SEM images, the second rows are optical microscope images, and the third rows are magnified SEM images for 20 and 100°C, respectively. Beachmark pattern is observed at 100°C due to a stick and slip effect. The two lines at the edge of a SWCNT line at 100°C form by a coffee ring effect.

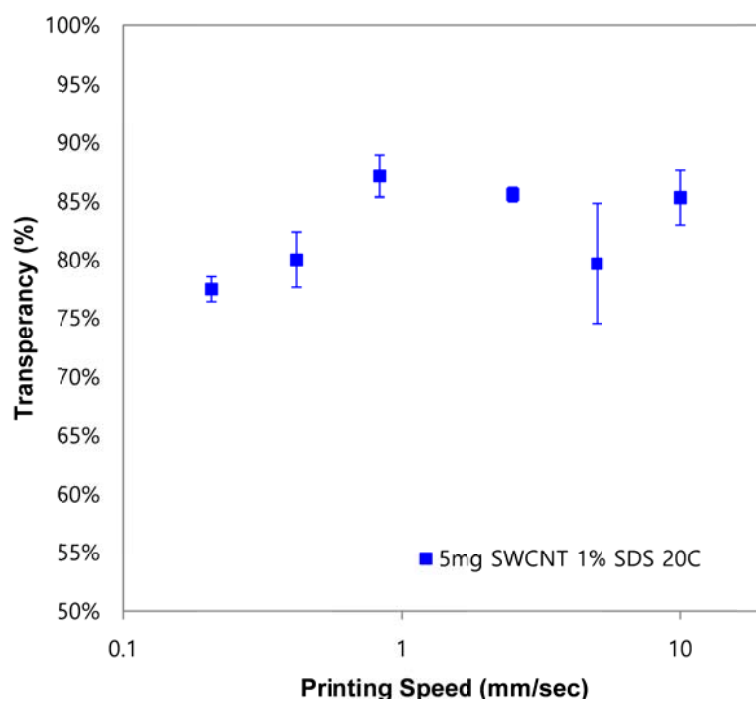


**Figure S6.** Transparency for a SWCNT line according to various printing speed at 20°C.

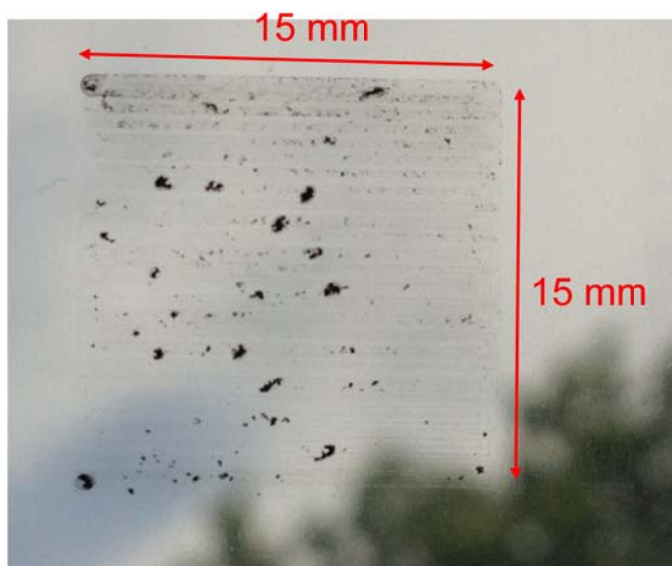


**Figure S7.** SWCNT film printed on a PET film (printing temperature: 80°C).

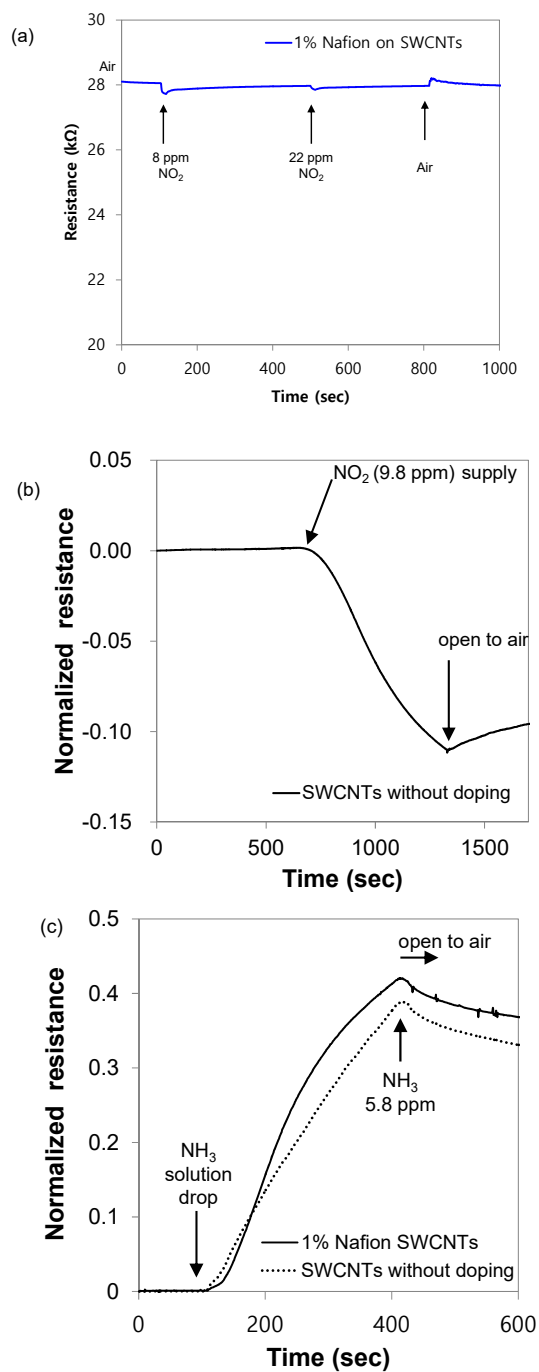


**Figure S8.** (a) Nafion-doped SWCNTs shows a negligible resistance change to NO<sub>2</sub> (b) Without doping, a SWCNT sensor shows 10.9% drop of the resistance. (c) When a 1.5% ammonia solution drop (4 $\mu$ L) was evaporated in a 4.8L chamber, SWCNT sensors with and without nafion doping show resistance increase by 41.7 and 38.7%, respectively. At the peak, the concentration was 5.8 ppm using a commercial ammonia sensor (MQ-135). In calculation, when the drop is completely evaporated, the concentration is 9.8ppm, which is higher than 5.8ppm due to ammonia absorbed on the chamber wall.

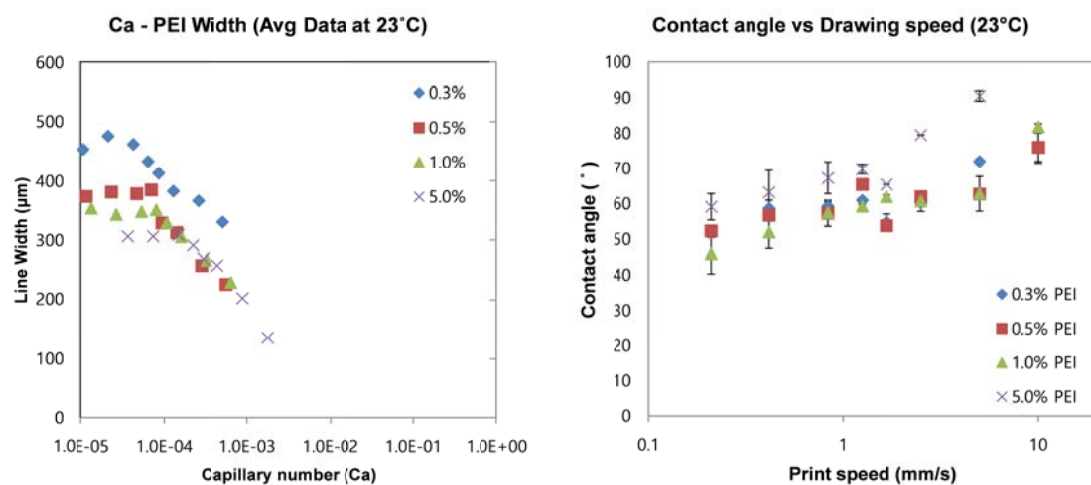


**Figure S9.** Line width for various concentrations of PEI diluted in deionized water.