Supporting Information

Quaternized Polymer–Single-Walled Carbon Nanotube Scaffolds for a Chemiresistive Glucose Sensor

Saniye Soylemez[†,‡], Bora Yoon[†,§], Levent Toppare[‡], and Timothy M. Swager[†,*]

[†]Institute for Soldier Nanotechnology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, United States

[‡]Department of Chemistry, Middle East Technical University, Ankara 06800, Turkey

[§]Nanomaterials Science Team, U.S. Army Natick Soldier Research, Development and Engineering Center (NSRDEC), Natick, Massachusetts 01760, United States

*Corresponding author: tswager@mit.edu

Table of Contents

Experimental

Materials

All chemicals and reagents were purchased from Sigma-Aldrich and used without additional purification, unless otherwise noted. SWCNTs (purified ≥95% as SWCNT, batch #UPT 1188-58BL) were obtained from Nano-C, Inc. Poly(4-vinylpyridine) (Mv = 200,000 g/mol) and 3-bromopropyltrichlorosilane (BPTS) were purchased from Scientific Polymer Products, Inc. and Gelest, respectively. ß-D-Glucose and gluconolactone were purchased from TCI and EMD Millipore Corp., respectively. 2-Bromoethanol (97%), D-fructose (99%), and sucrose (99%) were purchased from Alfa Aesar. Glucose oxidase (from Aspergillus Niger, 17300 units per g solid), glutaraldehyde (GA) as a cross linking agent and glucose assay kit (GAGO-20) were obtained from Sigma-Aldrich and used as received.

Characterization

ATR-FTIR spectra were recorded using a Thermo Scientific Nicolet 6700 FTIR with a Ge crystal for ATR. Raman spectra were collected with excitation at 633 nm laser using a Horiba LabRAM HR800 Raman spectrometer. Surface morphology of the device was investigated using a scanning electron microscope (SEM) (JEOL JSM-6700F FESEM), with an accelerating voltages of 3 and 10 kV. The static water contact angles on the surfaces before and after quaternization were measured using a Ramé-Hart goniometer (Model 590) by vertical deposition of 2 µL of water droplet. Glucose sensing experiments were performed using a Keithley 2400 source meter with a combination of Keithley KUSB-488B cable. Glucose sensing results were recorded using a

custom Labview program. In chemiresistive analyses, the data were given as the average of three measurements and standard derivations were recorded as ±SD. All measurements were performed at ambient conditions (25 ^{o}C).

Preparation of a P4VP-SWCNT Dispersion

To prepare the P4VP-SWCNT dispersion, the SWCNTs (5 mg) were first added into the solution of P4VP (*Mv* = 200,000 g/mol, 50 mg) in *N*,*N*-dimethylformamide (DMF, 10 mL), then the resulting mixture was sonicated for 1 h in an ultrasonic bath (Branson, 3510) chilled with ice. Finally, the dispersion was allowed to reach room temperature, and the suspension was centrifuged for 30 min at 15,000 *g* and allowed to stand overnight undisturbed as reported in the literature.[1] The supernatant was carefully removed from the mixture and directly used for the device fabrication via spray coating.

Fabrication of a Quaternized P4VP-SWCNT Scaffold

Glass substrates were cleaned and prepared according to a literature procedure.[2] First, the glass substrates (VWR Microscope Slides) were cleaned using acetone in an ultrasonic bath for 5 min followed by UV-ozone treatment using a UVO cleaner (Jelight Company Inc., Model 42) for 20 min. The cleaned glass substrates were fitted with a home-made stainless steel mask, and then layer of chromium (10 nm) and a subsequent layer of gold (120 nm) (99.99%, R.D. Mathis) were deposited onto the glass substrates using a thermal evaporator (Angstrom Engineering). Each device contains a gold pattern of electrode pair on the glass substrate. The gap between gold electrodes is 1 mm. Before the surface modification with BPTS, the glass substrates were cleaned using acetone and isopropyl alcohol sequentially in an ultrasonic bath for 15 min to remove dust. Then, the glass substrates were treated with piranha solution (a mixture of sulfuric acid and 30% hydrogen peroxide (1:1, v/v)) for 1 h followed by cleaning and drying with distilled water and nitrogen after each step, respectively. For 3-bromopropyltrichlorosilane (BPTS) modification, these cleaned glass substrates were incubated in anhydrous toluene containing 10% of BPTS solution at room temperature under argon atmosphere overnight. This modification was used to react with the P4VP-SWCNT to provide a surface anchor and excess BPTS was removed by washing the electrodes with pure toluene followed by drying with N_2. After drying of substrates, they were thermally annealed at 130 ^{o}C for 1 h. To produce a P4VP-SWCNT film a homemade transparency film (CG3700, 3M) mask was used to deposite the material selectively onto the gaps between electrode pairs, and the dispersion was manually spray coated with 400 µL of P4VP-SWCNT dispersion using an air brush (Revolution BR, Iwata). The rate of deposition was fixed as 40 µL/min at a distance of 10 cm with the substrate placed on a 130 ^{o}C hot plate under N_2 carrier gas of 2 bar pressure to obtain homogenous surface. After this process, following procedures were applied to obtain final substrate: The substrate was thermally annealed at 130 ^{o}C overnight to ensure the quaternization reaction between the surface bound alkyl bromides and pyridyl groups. Excess polymer and materials not covalently bound to the surface are removed by sonication in pure dichloromethane (DCM) for 1 min followed by drying under under N_2. For the quaternization reaction with 2-bromoethanol, 1 mL of 2-bromoethanol (97%) was added into the 4 mL of acetonitrile (ACN), and mixed well. Then, the modified device was placed into the ACN solution and then heated at 30 ^{o}C for 24 hours. Finally, the device was rinsed with pure ACN, and dried under a stream of N_2.

Fabrication of a P4VP-SWCNT-GOx Chemiresistive Sensor

Following the surface modification, the device was rinsed with distilled water and dried under N_2. Then 2 µL of glucose oxidase solution (10 µL of 2.5 mM pH 7.0 phosphate buffer solution containing 30.27 U GOx) was electrostatically immobilized onto the quaternized P4VP-SWCNT surface. GOx was chosen as the model enzyme. After 2 min, 2 µL of glutaraldehyde (GA) solution (1%) was added on the surface and allowed to dry in ambient air for 2 h to finalize the fabrication of the robust P4VP-SWCNT-GOx devices. The final resistance across the SWCNT network was between 350 kΩ -1 MΩ as measured by a multimeter. Devices were thoroughly washed with distilled water prior to use in order to remove unbound enzyme molecules. The use of GA not only provides intermolecular cross linking in proteins but also enhances anchoring of the enzyme molecules on the supporting material.[3]

Glucose Detection Measurement

Chemiresistive measurements with the devices were carried out at room temperature in the reaction cell containing 10 mL of NaOAc (2.5 mM, pH 5.5) by applying 0.1 V potential. Under the constant potential, the resistance change was monitored and recorded using a custom Labview program with the combination of Keithley 2400 Source Meter. The change in resistance resulted from exposure to glucose was calculated using the equation of $\Delta R\ (\%) = (R_0 – R)/R_0 \times 100\%$, R_0 is the initial resistance of the P4VP-SWCNT-GOx, and R is the resistance after exposure to glucose. After the background reached at a steady state, a certain amount of glucose was injected in the reaction cell and the resistance change was recorded as the sensor response. All experiments were carried out at ambient conditions. For consecutive measurements on a single device, the working buffer was refreshed and the device was rinsed with distilled water after each measurement.

Glucose oxidase (GOx) is a well-known oxido-reductose enzyme that catalyses the conversion of glucose and oxygen to give gluconolactone and hydrogen peroxide as follow:[4,5]

$$\beta\text{-D-Glucose} + O_2 \rightarrow \text{D-Glucono-1,5-lactone} + H_2O_2$$

The quantification of glucose can be monitored via the detection of enzymatically liberated hydrogen peroxide under constant potential. When the reaction occurs each glucose produces an equivalent of hydrogen peroxide and subsequently alters the resistance of the system. As desired, in the absence of GOx, no change in resistance was observed. We hypothesize that SWCNT based chemiresistive response to hydrogen peroxide produces a selective and sensitive glucose sensor. Moreover, this sensor showed different resistance changes upon exposure to different concentration of glucose. Following the optimization and characterization studies, the P4VP-SWCNT-GOx device was tested for glucose in beverage samples that were used without any pretreatment. Experimental analyses were achieved in the three replicates, and data were calculated as the mean ± SD.

Raman Spectroscopy

Oxidation of CNTs by H_2O_2 was also a central topic when using CNTs as H_2O_2 sensors or sensitive glucose sensors based on oxidase[6,7]. The oxidation of CNTs with H_2O_2 causes defects on the nanotube structure. To explain the reason of the increase in the Raman disorder signal of SWCNT by H_2O_2, we prepared a device with P4VP-SWCNT not containing GOx and obtained its Raman spectrum after exposure to an appropriate concentration of H_2O_2 for 20 times. Figure S-2(A) shows the Raman spectra of the P4VP-SWCNT before and after H_2O_2-treatment (20 times exposure). The results revealed that after exposure to H_2O_2, there was a slight increase in the D/G band ratio from 0.05 to 0.07. This is an evidence of the defect by H_2O_2, which has been reported in other studies.[8,9] The structure distribution of semiconducting SWCNTs has been strongly modified by the peroxide treatment. To test the effect of the gluconolactone, which is the product of the enzymatic reaction, Raman spectra were taken for the P4VP-SWCNT-GOx after exposure to an appropriate concentration of gluconolactone and H_2O_2 for 20 times each. In Figure S-2(B), the red line shows the Raman spectrum of the gluconolactone-treated P4VP-SWCNT-GOx with the D/G band ratio of 0.06 while the one exposed to H_2O_2 for 20 times showed a notable increase in the D/G band ratio of 0.15 (Figure S-2(B), blue line). Interestingly, we found that the enzymatic reaction caused a huge increase in the D/G band intensity (0.56) after exposure to glucose (Figure 3B). We presume that there is a synergetic effect of the enzyme that affects the electronic nature of the SWCNT under hydrogen peroxide. GOx is flavoprotein, containing two tightly bounded flavine adenine dinucleotide redox centers. The redox centers lead an electron transfer during the enzymatic reaction. The GOx from *Aspergillus niger* catalyzes the oxidation of β-glucose to D-glucono-δ-lactone and hydrogen peroxide with molecular oxygen. Presumably, this reaction may affect the electronic structure of the nanotube. Moreover, the oxidation process of β-glucose to D-glucono-δ-lactone may disturb the π electronic states in the SWCNT sidewalls.

References

(1) Yoon, B.; Liu, S.F.; Swager, T.M. Surface-Anchored Poly(4-vinylpyridine)–Single-Walled Carbon Nanotube–Metal Composites for Gas Detection. *Chem. Mater.* **2016**, 28, 5916-5924.

(2) Frazier, K.M.; Swager, T.M. Robust Cyclohexanone Selective Chemiresistors Based on Single-Walled Carbon Nanotubes. *Anal. Chem.* **2013**, *85*, 7154-7158.

(3) Gouda, M.D.; Kumar, M.A.; Thakur, M.S.; Karanth, N.G. Enhancement of Operational Stability of an Enzyme Biosensor for Glucose and Sucrose Using Protein Based Stabilizing Agents. *Biosens. Bioelectron.* **2002**, *17*, 503-507.

(4) Besteman, K.; Lee, J.O.; Wiertz, F.G.M.; Heering, H.A.; Dekker, C. Enzyme-Coated Carbon Nanotubes as Single-Molecule Biosensors. *Nano Lett.* **2003**, *3*, 727-730.

(5) Liu, Y.; Feng, X.; Shen, J.; Zhu, J.J.; Hou, W. Fabrication of a Novel Glucose Biosensor Based on a Highly Electroactive Polystyrene/Polyaniline/Au Nanocomposite. *J. Phys. Chem. B.* **2008**, *112*, 9237–9242.

(6) Lim, S.H.; Wei, J.; Lin, J.; Li, Q.; You, J.K. A Glucose Biosensor Based on Electrodeposition of Palladium Nanoparticles and Glucose Oxidase onto Nafion-Solubilized Carbon Nanotube Electrode. *Biosens. Bioelectron*. **2005**, 20, 2341-2346.

(7) Zhu, Y.N.; Peng, T.Z.; Li, J.P. Electrocatalytic Oxidation of Hydrogen Peroxide at a Glassy Carbon Electrode Modified with Platinum-Decorated Carbon Nanotubes. *Chem. J. Chin. UniV.* **2004**, 25,1637-1641.

(8) Miyata, Y.; Maniwa, Y.; Kataura, H. Selective Oxidation of Semiconducting Single-Wall Carbon Nanotubes by Hydrogen Peroxide. *J. Phys. Chem. B* **2006**, 110, 25-29.

(9) Peng, Y.; Liu, H. Effects of Oxidation by Hydrogen Peroxide on the Structures of Multiwalled Carbon Nanotubes. *Ind. Eng. Chem. Res.* **2006**, 45, 6483-6488.

Supporting Figures and Table

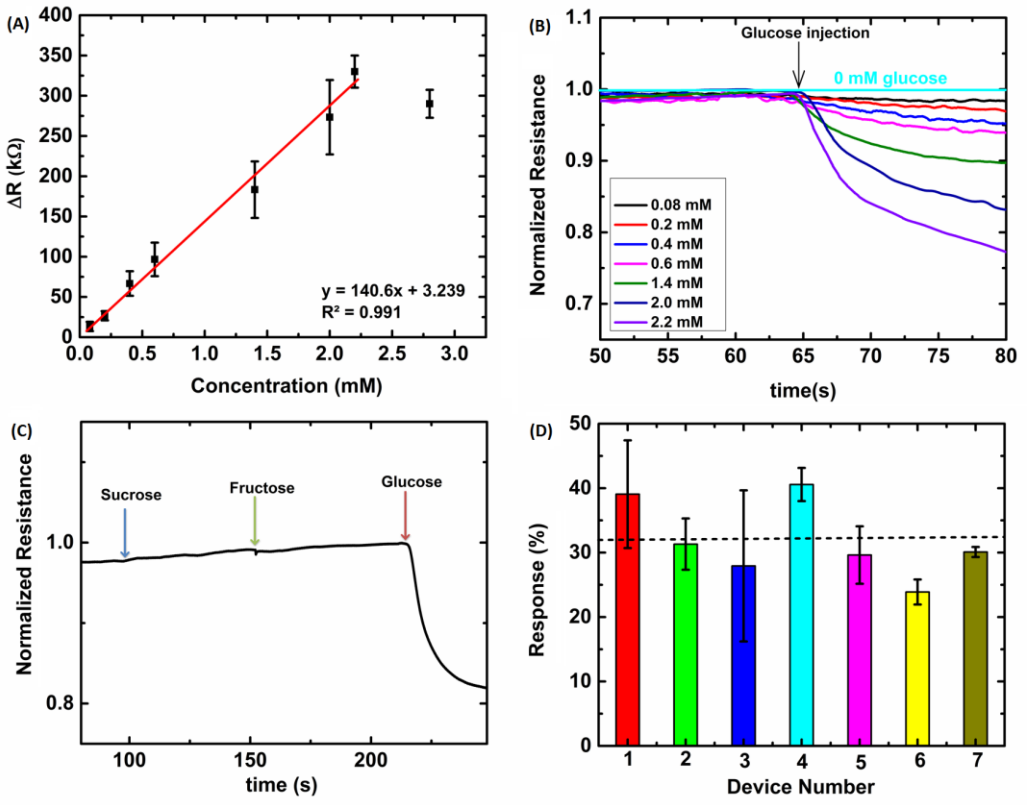

Figure S-1. (A) Calibration curve for glucose (in 2.5 mM NaOAc buffer, pH 5.5) (error bars show three consecutive measurements with the same device), (B) The chemiresistive device response to various glucose concentrations between 0.08 and 2.2 mM, (C) Sensor selectivity for different sugars (2.0 mM in 2.5 mM NaOAc buffer, pH 5.5), and (D) Responses of glucose using different devices under the optimum conditions: The dotted line indicates the average value of the devices (error bars show three consecutive measurements with the same device).

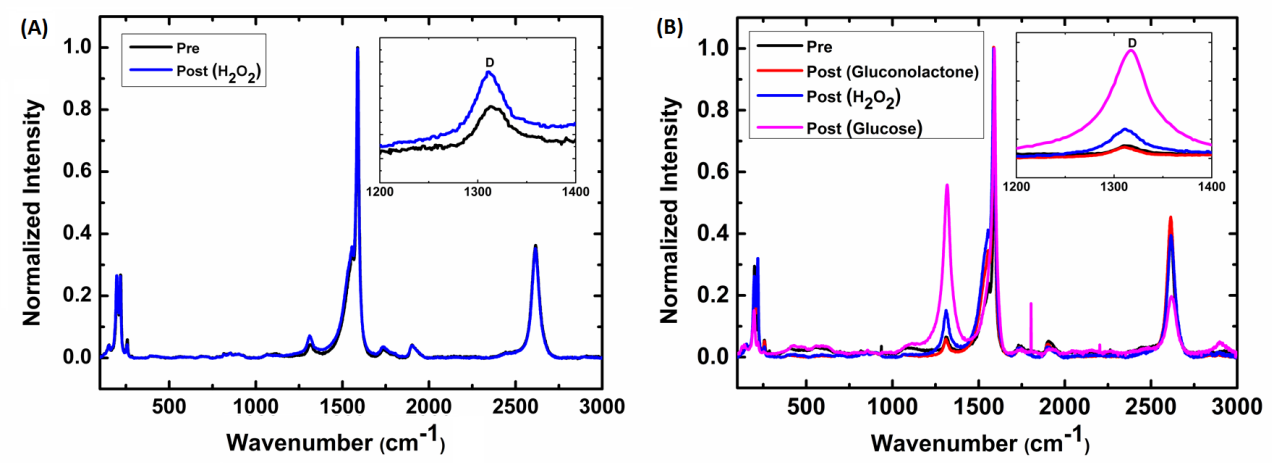

Figure S-2. Raman spectra of (A) P4VP-SWCNT before and after exposure to H_2O_2 (20 times exposure), and (B) P4VP-SWCNT-GOx before and after exposure to gluconolactone, H_2O_2 and glucose (20 times exposure) in 2.5 mM NaOAc buffer solution.

Table S-1. Determination of glucose in beverage samples.

Glucose (mol/L) Sample	Spectrophotometric Assay	Chemiresistive Sensor	% Error
S fruit punch juice	0.204	0.231	13.23
L ice tea	0.130	0.125	-3.84