Electronic Supplementary Information (ESI)

2. Experimental

2. 1. Materials

A conducting polymer, 2,2′:5′,2′′-terthiophene-3′(*p*-benzoic acid) (TTBA) was synthesized through the Paal-Knorr pyrrole condensation reaction.[1] Tetrabutylammonium perchlorate (TBAP, electrochemical grade) was purchased from Fluka (USA) and purified according to a general method, followed by drying under vacuum at 1.33×10^{-3} Pa. 1-Ethyl-3-(3-(dimethylamino)-propyl) carbodiimide (EDC), *N*-Hydroxysuccinimide (NHS), dichloromethane (99.8%, anhydrous and sealed under N_2 gas), and trisodium citrate were purchased from Sigma Co. (USA). Sodium tetrahydridoborate and $HAuCl_4 \cdot 3H_2O$ were obtained from Aldrich (USA). The aptamer derived in SELEX [2]; modified with an amine group at the 5′ position was used in the present study (NH_2-5′-GGGAATTCGAGCTCGGTACCATCTGTGTAAGGGGTAAGGGGTGGGGGTGGGTAC GTCTAGCTGCAGGCATGCAAGCTTGG-3′). The PAGE purified aptamer was purchased from Bioneer (S. Korea). HPLC grade DAN was purchased from Sigma-Aldrich (USA) and stored at 4 ^{o}C. The aptamer stock solution was prepared in autoclaved Tris EDTA buffer (10.0 mM Tris-HCl and 1.0 mM EDTA) of pH 8.0 and stored at -20 ^{o}C. RPMI 1640 medium, Dulbecco's Modified Eagle's Medium (DMEM), human serum, fetal bovine serum (FBS), trypsin-EDTA, penicillin/streptomycin, Hank's balance salt (HBS) solution, were purchased from Sigma-Aldrich. All aqueous solutions were prepared in double distilled water obtained from a Milli-Q water purifying system (18 MΩ cm).

2. 2. Apparatus

All electrochemical experiments were performed in a three-electrode cell. A modified electrode (area = 0.07 cm^2) was used as the working, Ag/AgCl in saturated KCl as the reference, and Pt wire as the counter electrodes. Impedance spectra were recorded with the EG&G PAR 273A potentiostat/galvanostat and a lock-in amplifier (PAR EG&G, Model 5210) linked to a personal computer. The frequency was scanned from 100.0 kHz to 100.0 MHz at the open circuit voltage, acquiring five points per decade. The amplitude of sine voltage of 10.0 mV was used. SEM images were obtained with a Cambridge Stereoscan 240. XPS was

performed using a VG Scientific XPSLAB 250 XPS spectrometer and a monochromated Al Kα source with charge compensation. An Au-coated working electrode (area: 0.196 cm^2; 9.0 MHz; AT-cut quartz crystal) was used for the QCM experiment.

2. 3. Fabrication of the sensor probe

The schematic of the biosensor probe fabrication is presented in Scheme. 1. AuNPs were electrodeposited onto the GCE before the polymerization of TTBA in a 0.5 M H_2SO_4 solution containing 0.001% $HAuClO_4$ using linear sweep voltammetry (LSV) from 1.5 to 0.4 V. The electrodeposition conditions were as follows: 60.0 s deposition time, -1.0 V deposition potential, 100.0 mV/s scan rate, and potential cycling three times. The pTTBA layer was formed on the GC/AuNPs through electropolymerization of 1.0 mM TTBA monomer in a 0.1 M TBAP/CH_2Cl_2 solution by a one-time potential cycling from 0.0 to +1.4 V at 100.0 mV/s. After polymerization, the carboxylic acid groups of the pTTBA layer were activated with EDC/NHS and aptamer was immobilized to form the GC/AuNPs/pTTBA/aptamer. Thereafter, the modified electrode was washed with the same buffer followed by 0.1 M mercaptoethanol for 1 min to remove any unbound aptamer. The formation of each layer was examined by XPS and aptamer immobilization was also quantified using quartz crystal microbalance (QCM) experiments.

2. 4. Cells sample preparation

The HeLa (human cervical cancer), MCF-7 (human breast adenocarcinoma) HT-29 (human colon carcinoma), and SK-BR-3 (human breast adenocarcinoma), ovarian surface epithelial (OSE) cells, MCF-10A, and HEK-293 cell lines were obtained from American Type Culture Collection (Manassas, VA, USA). The HeLa and MCF-7 cell lines were cultured in DMEM medium while HT-29 and SK-BR3 cell lines were cultured in RPMI 1640. All noncancerous cells were cultured in DMEM medium. The cells were grown at 37 ^oC in 5% CO_2 atmosphere in the appropriate medium supplemented with a 10% heat-inactivated fetal bovine serum, 100.0 units/mL of penicillin, and 100.0 units/mL of streptomycin. The medium was replaced every second day. All cancerous and noncancerous cells were washed with HBS solution to remove culture medium. Cancer and noncancerous cells were treated with 1.0 μM DAN in PBS (pH 7.4) for 20 mins in an ice bath at 4 ^oC to prevent internalization of DAN.[3] The cells were then centrifuged at 1000 rpm to remove the unadsorbed DAN from the cell surfaces. Similar kind of experiment was performed under the same experimental conditions without DAN as a positive control experiment. DAN treated and untreated cells were incubated with sensor probe. In another set of control experiment

GC/AuNPs/pTTBA modified electrode (without aptamer) was incubated with cells. All modified surfaces were tested for electrochemical impedance spectroscopy (EIS) immediately after cell interaction.

2. 5. Analytical procedures

An electrochemical cell for the EIS analysis was a three-electrode configuration that consists of an Ag/AgCl reference electrode in saturated KCl solution, a platinum counter electrode, and GC/AuNPs/pTTBA/aptamer as working electrode. Faradaic impedance spectra were recorded using a PARSTAT 2263 (USA) at an open circuit potential of 100.0 kHz to 100.0 MHz. All electrochemical impedance measurements were performed in a PBS (pH 7.4) buffer and impedance spectra were collected as a form of Nyquist plots.

3. Results and discussion

3. 1. Characterization of the sensor probe

Foremost, the AuNPs were electrodeposited onto the glassy carbon electrode (GCE) in a 0.5 M H_2SO_4 solution containing 0.001% $HAuClO_4$ using linear sweep voltammetry (LSV) from +1.4 to +0.5 V. Next, a 2,2′:5′,2′′-terthiophene-3′(*p*- benzoic acid) (TTBA) was polymerized on the GC/AuNPs surface by electropolymerization from a 1.0 mM monomer TTBA containing a 0.1 M TBAP/CH_2Cl_2 solution. The monomer oxidation peak appeared at +1.2 V during the anodic from 0.0 to +1.4 V at the scan rate of 50.0 mV/s. There was a distinct reduction peak at +0.8 V in the reverse cathodic scan from +1.4 V, corresponding to the reduction of the oxidized polymer film formed on the GC/AuNPs surface. Larger redox peak currents were observed at GC/AuNPs compared to those from the polymerization at bare GC, indicating that the deposition of AuNPs before the TTBA film increases the electrical property of the sensor surface. The NH_2 terminated DAN selective aptamers was immobilized to form a GC/AuNPs/pTTBA/aptamer sensor probe which was confirmed through XPS and QCM.

To confirm the formation of each sensor probe layer, we characterized the sensor probe using XPS. The XPS spectra were calibrated with a C1s peak at 284.6 eV as an internal standard. Fig. S1 contains the XPS spectra for the surfaces of (a) GC/AuNPs/pTTBA and (b) GC/AuNPs/pTTBA/aptamer. The successful electrodeposition of AuNPs on the sensor surface was confirmed by the observation of two sharp peaks at 86.5 and 84.6 eV (data not shown). The TTBA coated electrode showed an S2p peak at 163.8 eV corresponding to the C-S bond that was due to the sulfur component of pTTBA, however, the peak at 163.8 eV was

not observed for the GC/AuNPs sensor surface. The N1s peak for the aptamer immobilized surface was observed at 398.8 and 399.8 eV, which corresponds to the C-N, -NH, and O=C-NH- bonds respectively, because of the covalent bond formation between NH_2 groups of aptamer and -COOH groups of pTTBA. The aptamer immobilized electrode yield a very clear P2p peak at 131.9 eV, which corresponded to phosphate, the major structural component of the aptamer backbone, indicating the successful immobilization of the aptamer. The quantitative immobilization of aptamer was also evaluated by the QCM experiment onto the pTTBA film based on the frequency change. In the case of aptamer immobilization, the decrease in the frequency reaches a complete steady state after about 1 h, with an overall frequency change (Δf) of 0.11 kHz, corresponding to a mass change (Δm) of 132.8 ± 7.2 ng based on a previously defined equation. [4] The number of molecules of aptamer on the electrode surface was determined to be 1.7 x 10^{-11} mol/cm^2. These results reconfirm the successfully immobilization of aptamer onto the pTTBA layer.

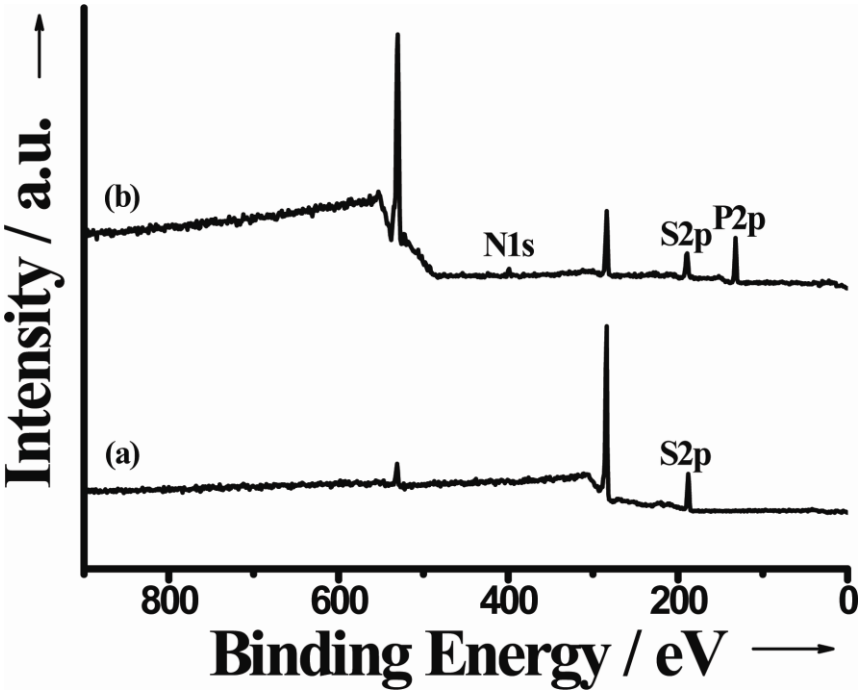

Fig. S1 XPS characterization of probe

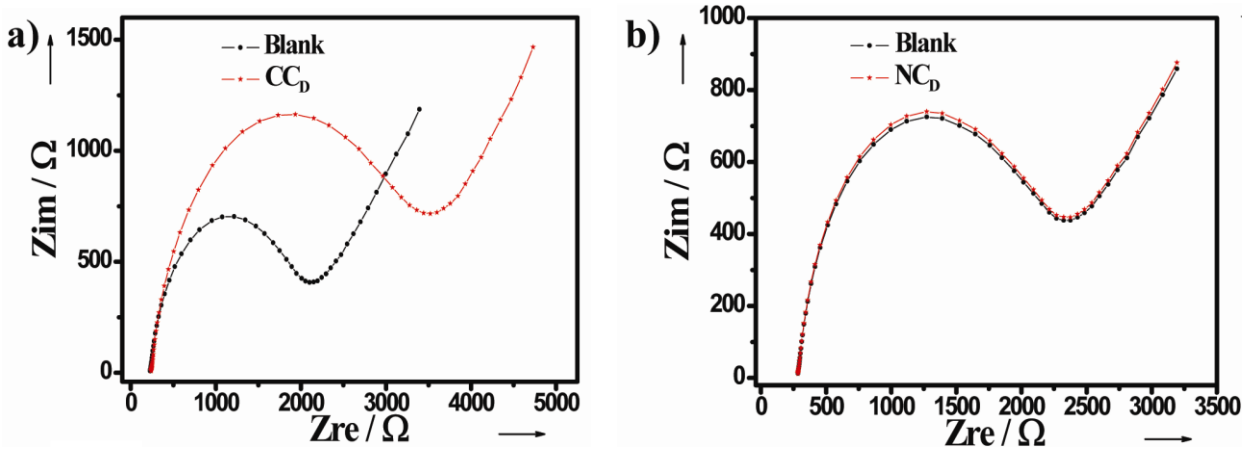

Fig. S2. Nyquist plot for the response of a) CC_D (HeLa) and b) NC_D (OSE) reacted at GC/AuNPs/pTTBA/aptamer sensor in 0.1 M PBS (pH 7.4)

References

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