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SWCNTs based Aptasensor System for Antibiotic Oxytetracycline Detection in Water Samples

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Abstract: Oxytetracycline (OTC) is a member of the broad-spectrum tetracycline (TC) group of antibiotics and TCs are widely used to prevent bacterial infections in livestock and increase their growth rate. Hence a large percentage of the antibiotics is either accumulated in tissues or excreted and released into the environment that leads to serious health implications such as antibiotic resistance. Thus, simple, fast and easy to use methods are needed for OTC detection. Here a simple and highly sensitive aptamer-based single walled carbon nanotubes (SWCNTs) biosensor containing probe-DNA immobilized on functionalized SWCNTs was developed for fast and specific OTC detection. We employed a newly developed flexible biosensor device which fabricated by high-rate nanoscale offset printing process using directed assembly and transfer of SWCNT. Employing simple directed assembly and non-covalent functionalization process these

fabricated probe DNA-based SWCNTs biosensors were designed with two electrode terminals to allow continuous resistance response monitoring for the antibiotics detection. The developed environmental sensor had detection range of 10 μg/L to 75 μg/L (20~325 nM), with detection limit of 1.125 μg/L (2.5 nM). When compared to other detection methods such as colorimetric [1], electrical [2] or cantilever [3] based biosensor systems, the biosensor developed here is simpler and faster (less than 10 minutes, including pre- incubation, measurement and regeneration) with lower detection limit. And the portable platform also allows for potential on-site or real-time measurements. The biosensor could be regenerated and reused for over 20 times with good stability with signal decrease less than <15%. In addition, its inherent miniature size makes this biosensor potentially useful for simple potable model for environmental and industrial applications.

Keywords; Aptamer, Oxytetracycline, Environmental sensor, Antibiotics detection, Carbon nanotube, SWCNTs.

INTRODUCTION

Oxytetracycline (OTC) is a member of the broad-spectrum tetracycline (TC) group of antibiotics and TCs are widely used as a veterinary antibiotic and an animal growth promoter [3]. Excessive use and abuse of TCs in farm animals can cause accumulation of antibiotics in food products, including meat, milk and chicken eggs[4, 5]. Consequently, a large percentage of the antibiotics is either accumulated in tissues or excreted and released into the environment via manure or other discharges from aquaculture [6]. Presence of TCs in the environment leads to serious implications for human health such as contributing to antibiotic resistance phenomena.[7] Therefore, several countries have set maximum residue limits (MRLs) of antibiotics for many food products[8]. Thus, effective analytical methods for the detection of a trace amount of OTC in the environment is of great need.

Traditionally, chromatography methods, including high-performance liquid chromatography (HPLC) and liquid chromatography-tandem mass spectrometry (LC–ESI-MS/MS), have been used for the detection of TCs in food products[9, 10]. These methods provide accurate detection of TCs, however, they demand expensive equipment, tedious sample extraction procedures, and expert technical skills. As alternatives, several biosensor systems including immunochemical methods using have been demonstrated with features of simplicity, cost-effectiveness, high sensitivity and specificity [11]. In one of the good examples of resent immuno based OTC detection, monoclonal antibody provided high specificity with a visual detection limit of 2 ng/mL [12]. However, most of the limitations of immunochemical biosensor such as lack of stability and reusability is the reliance on usage of antibodies, which is described here briefly with the comparison with aptamers and other methods (table 1).

Another type of immune specific recognition agent-nucleic acid based aptamer, has been shown to be an excellent alternative to antibody. Aptamers are short single-stranded oligonucleotides with a three-dimensional structure that show high affinity binding and high-specificity target recognition [13, 14]. Aptamers have a number of advantages over antibodies because they are small and can be designed against any type of target, including toxic compounds or poor immunogenic targets [15], and aptamers are much flexible than protein compounds, the binding can result in large structural changes of aptamers [16, 17]. In addition, a variety of derivatives such as labeled molecules can be conveniently attached at the 3′ or 5′ end of an aptamer without affecting the target-binding site[18, 19,20, 21]. Moreover, aptamers also have many other advantages including high binding affinity, simplicity of synthesis, ease of labeling, and excellent stability. Aptamers and several antibiotics belonging to the targeting tetracycline class have been used for the development of biosensors, including the detection of OTCs in many food products from animals [22,23].

In recent years, many ssDNA (single strained DNA) aptamers that selected by the Systematic Evolution of Ligands using the Exponential Enrichment (SELEX) process have been reported for various small molecular targets [20, 21]. Aptamers and several antibiotics belonging to the targeting tetracycline class have been used for the development of biosensors, including the detection of OTCs in many food products from animals. For example, as listed in table 1, fluorescent switch based aptasensor showed a lower limit of detection (LOD, 1,67 nM) and short detection time[24]. In another example, colorimetry has been proposed as a simple technique for the detection of signaling with the naked eye on-site with detection limit comparable to UV/vis spectrophotometer analysis for OTC detection[25]. Meng et all reported an aptasensor based

ultrasensitive surface enhanced Raman scattering sensor that reached the lowest LOD for oxytetracycline detection with fM level detection range [26].

Recently, nanowires, and nanotubes, nanosphere as donors of electrical response have been researched to realize the minimized nanostructures in a field of biosensors. Nanoscale of biosensors device supports in-vivo system, high sensitivity, and low limit concentration of detection[27]. Researches of nanoscale biosensors try to obtain the ease detections, such as label-free, rapid, low-cost, and multi-detections. Miniaturized biosensors are required to detect and quantify small molecules with high sensitivity, selectivity, and stability. Here, we are using a flexible biosensor device which fabricated by high-rate nanoscale offset printing process using directed assembly and transfer of nanomaterials. With this technique, single walled carbon nanotubes (SWCNTs) were assembled at the desired locations with controllable high density and good uniformity by controlling assembly parameters, which leads us to develop more stable and reusable biosensor system. This is the first nano-biosensor reported in the literature that uses directly assembled SWCNT for OTC detection in real environmental samples. We have overcome several challenging limitations associated with nano-sensor such as unstable, non-reproducible sensing performance due to the uncontrollable and disorganized SWCNTs assembly structure, as well as the high cost and complicated assembly procedure such as CVD (details can be found in the supplementary information folder). Compared to previously reported SWCNT-based biosensors that use FETs (field effect transistors), our system is simpler and cost effective in fabrication steps and has quite similar sensitivity and fast response capability with FETs. Thus, the developed SWCNTs based nano-biosensor system is quite suitable for sensitive, rapid and cost effective environmental pollutants detection. In resent works, various techniques of electrode modification have been utilized for the immobilization of biomolecules onto SWCNTs with

covalent or physical bonding methods[28]. Physical (non-covalent bonding) method using π-π stacking can maintain the chemical characteristics while covalent bonding utilizes chemical forces to immobilize materials onto SWCNTs, which enable to change chemical properties[28-30].

In this study, a novel aptamer-based SWCNTs biosensor was developed for OTC detection in environmental samples using an indirect competitive mode sensing mechanism. The indirect detection mode was adopted to overcome the problem with non-specific binding and adsorption caused by the environmental water sample matrices [31]. Additionally, indirect detection mode with non-immobilized aptamers provides much more relax binding between OTC and aptamers, and shorter binding time [32]. The biosensor's sensing time, sensitivity, specificity, resistance to background interference and reusability were evaluated. The developed OTC sensing system exhibits a sensitive response concentration range and detection limit comparable to OTC levels in environmental water (10 μg/L to 100 μg/L, depends on the area)[33-35] samples and therefore potentially applicable for easy-to-use and on-site analysis without any pre-concentration and treatment steps. The biosensor developed here is simpler and faster (less than 15 minutes, including pre- incubation, measurement and regeneration) compared to other developed systems in the literature (Table 1). And the miniature and portable platform also allows for potential on-site or real-time measurements.

Table 1. Performance Comparison of SWCNT Oxytetracycline biosensor with other biosensors and analytical methods reported in the literature for OTC detection.

Detection method	Detection Limit	Stability	Repeatability	Real sample application	Detection time	Ref.
Aptamer-Based Cantilever Array Sensors	1.0 nM	----	Tried 3 times	NA	20 mins.	[3]

Ultrasensitive SERS aptasensor	8.7 fM	----	-----	Fish meat samples	~ 1 hour	[26]
Nanoporous based Electrochemical Biosensor	10 nM – 40 mM	------	------	NA	Less than 1 hour	[36]
Sandwich based immunogold assay	4.0 nM	---	---	Fish tissues	3-4 mins.	[12]
Carbon-Dots-Based Lab-On-a-Nanoparticle	60 nM	----	----	real food samples	Less than 30 mins.	[37]
Aptamer-Based Fluorescent Switch	1.67 nM	----	----	Milk samples	~ 30 mins.	[24]
Indirect competitive assay-based aptasensor	25 nM	---	5 times	Milk samples	~ 1 hour	[38]
Molecularly imprinted based potentiometric sensor	50 μM	2 weeks	---	Urine samples	1-2 hours	[39]
Colorimetric aptasensor	25 nM	---	---	Raw milk sample	~ 1 hour	[25]
SWCNT aptamer biosensor	2.5 nM	30 days	22 Repeats	Real waste water samples	Less than 15 mins.	This study

MATERIALS AND METHODS

Reagents. Oxytetracycline (OTC) was purchased from Sigma-Aldrich (MO, USA). The linker; 1 - Pyrenebutanoic acid-succinimidyl ester (PBSE), was purchased from Invitrogen (CA, USA). Single-stranded DNA aptamer against OTC, which was isolated by SELEX process from a random ssDNA library with specific Kd value of 9.61 ± 0.3 nM [40], and probe-DNA were purchased from Integrated DNA Technologies (USA). The sequences for the aptamer and the aminated probe-

DNA are: 5'-GGAATTCGCTAGCACGTTGACGCTGGTGCCCGGTTGTGGTGCGAGTGT TGTGTGGATCCGAGCTCCACGTG-3 (aptamer), 5'- /5AmMC6/CACGTGGAGCTCGGATC CACACAACA -3' (Probe-DNA).

Both aptamer and probe DNA were dissolved in 100 mM PBS and kept frozen at -20ºC for storage. Buffer solution of 100 mM PBS (0 mM NaCl, 25 mM KCl, 10 mM $MgCl_2$ and pH 7.4) was used for dissolving all DNA sequences, OTC and water sample effluents. For sensor specificity evaluation, a number of antibiotics such as amoxicillin, diaminofen, genomiycin, amphotericin and ciprofloxacin (Thermo Fisher Scientific Inc. PA, USA) were tested.

Fabrication of Flexible SWCNT Biosensor System. The flexible biosensor was fabricated by directed assembly and offset printing transfer using reusable damascene template. The fabrication processes were described in the previous paper[41]. Briefly, a multiscale offset printing approach that enables the printing of nano-, micro-, or macroscale structures in minutes over small or large areas was described. The process starts with "inking" of patterns on specially fabricated reusable Damascene templates using electrophoretic directed assembly of nanomaterials from a suspension (ink) that contains nanoparticles (SWCNTs). This inking process is conducted at room temperature and pressure. The second step consists of "printing" where the assembled nanomaterials on the template are then transferred to another substrate. After the transfer process, the template is ready to be reused immediately in the next assembly and transfer cycle (figure S1, for detailed information please check supporting information file).

Electrochemical measurement. Electrochemical measurements of conductivity of each SWCNT biosensor were conducted using a probe station (4156C, Agilent Technologies Co., Ltd., USA) at an ambient condition. The electrical properties of the probe-DNA-modified SWCNTs devices during the introduction of OTC-aptamer was measured by meter probes (SE-TL, SIGNATONE,

USA) connecting with each source and drain of gold electrode. A source drain bias of 0~100 mV was maintained throughout the measurements of the electrical signal, and the pulse width was 1.0 s. The plates were cleaned thoroughly with PBS (pH 7.4) and DI water and dried with nitrogen gas after the electrical measurement of each sample.

Immobilization of Probe-DNA (complementary to OTC aptamer sequence) onto SWCNTs bridge Surface. The selection of PBSE as a linker for this study was based on its versatile functionality. It not only exhibits strong luminescence in solution but also is attached to CNTs through non-covalent π- π stacking that does not impact the geometric and electronic configuration of CNT [42]. Its aromatic hydrophobic domain spontaneously binds to the hydrophobic CNT sidewalls through non-covalent molecular adsorption. Furthermore, the π electrons were shown to enhance the electronic and thermal properties of CNT (Figure S2 and S3) [43, 44]. In addition, the hydrophilic domain of PSE, the succinimidyl ester group, provides amine reactive sites that can serve as binding sites of bio- or abio-ligands 20 for further applications to develop bio/abio hybrid systems.

For sidewall functionalization of CNT with PBSE, the TP/MWCNT electrodes were soaked in a PBSE solution [1-pyrenebutanoic acid succinimidyl ester-2 mg/ml in N,N-DMF (*N,N*Dimethylformamide)] for 2 h at room temperature, washed thoroughly with N,N-

DMF to remove excess PBSE and then with ID water (figure S3). The IV profile of the linker modified electrodes was observed (figure 3). Probe-DNA was dissolved in bicarbonate buffer (0.1 mM, pH 9.2) and then stored at -20 °C until use. For probe-DNA immobilization, PBSE-modified SWCNTs electrodes were incubated with 0.01 and 0.05 mg/ml probe-DNA for overnight at 4 °C. Excess probe-DNA was removed by washing with phosphate buffer and ID water. Each sensor electrode was tested immediately for I-V profile (figure 2).

Optimization of the Sensing Conditions. Sensing condition optimization studies were performed for various sensing steps. The incubation time length, aptamer concentration for the pre-mixing step and the probe-DNA concentration were optimized separately. A varying incubation time of 1, 3, 6 and 10 min was conducted and compared. Tests with a series of different aptamer concentrations (0.1, 0.5, 1.0, 10, 50, and 100 μg/L) were performed to determine the optimal aptamer concentration. Additionally, two different probe-DNA concentrations were immobilized onto the SWCNTs surface to conclude the more appropriate one for the sensing performance.

Evaluation of the SWCNT OTC biosensor Specificity. To determine the specificity of the aptamer biosensor for detecting OTC, a number of antibiotics such as amoxicillin, diaminofen, genomiycin, amphotericin and ciprofloxacin were evaluated. The biosensor system's

responses for these chemicals were compared with the results of OTC detection and control experiment (100 µg/L OTC-aptamer without any antibiotics).

Analysis of Spiked environmental water Samples. To evaluate the potential matrix effect of real environmental water sample on the sensor performance, we analyzed spiked samples that contained different concentrations of OTC (10 µg/L and75 µg/L) in representative real environmental water samples; wastewater effluent representing the most "dirty" water and tap water representing a "clean" water sample. This approach is widely accepted in the literature [45-48]. The wastewater effluent samples were filtered through 0.22 µm filters to remove all particulates before they were spiked with OTC. Three independent experiments were performed for all samples. Similar analytical procedures were followed as described above.

RESUTLS AND DISCUSSIONS

SWCNT OTC biosensor sensing mechanism. The sensing mechanism of the SWCNTs aptamer-based biosensor for detection of OTC is represented in Figure 1. We employed an indirect [46]concentrations of OTC with a fixed amount of OTC-aptamer (see details in sensor optimization section). Upon the completion of binding between OTC and its

specific aptamer, the remaining free aptamers concentration is inversely proportional to that of OTC in the water sample. The sample mixture is then injected through the gold chip surface; the remaining free aptamers are allowed to bind to the immobilized probe-DNA which is complementary to a certain section of the OTC-aptamer (reaction time of 3 min). The I-V signal was recorded before and after OTC + aptamer mixture injection onto the sensor surface and resistance (R) differences were observed for each experiment.

$\Delta R/R_0$ values were calculated for each experiment, where

$\Delta R = R_s - R_0$

R_0= Resistance measured as background before sample injection

Rs= Resistance measured after sample injection

To reuse the sensor, the sensing surface was regenerated with a 0.5% SDS solution for 5 min and washed with a PBS solution (pH 7.2).

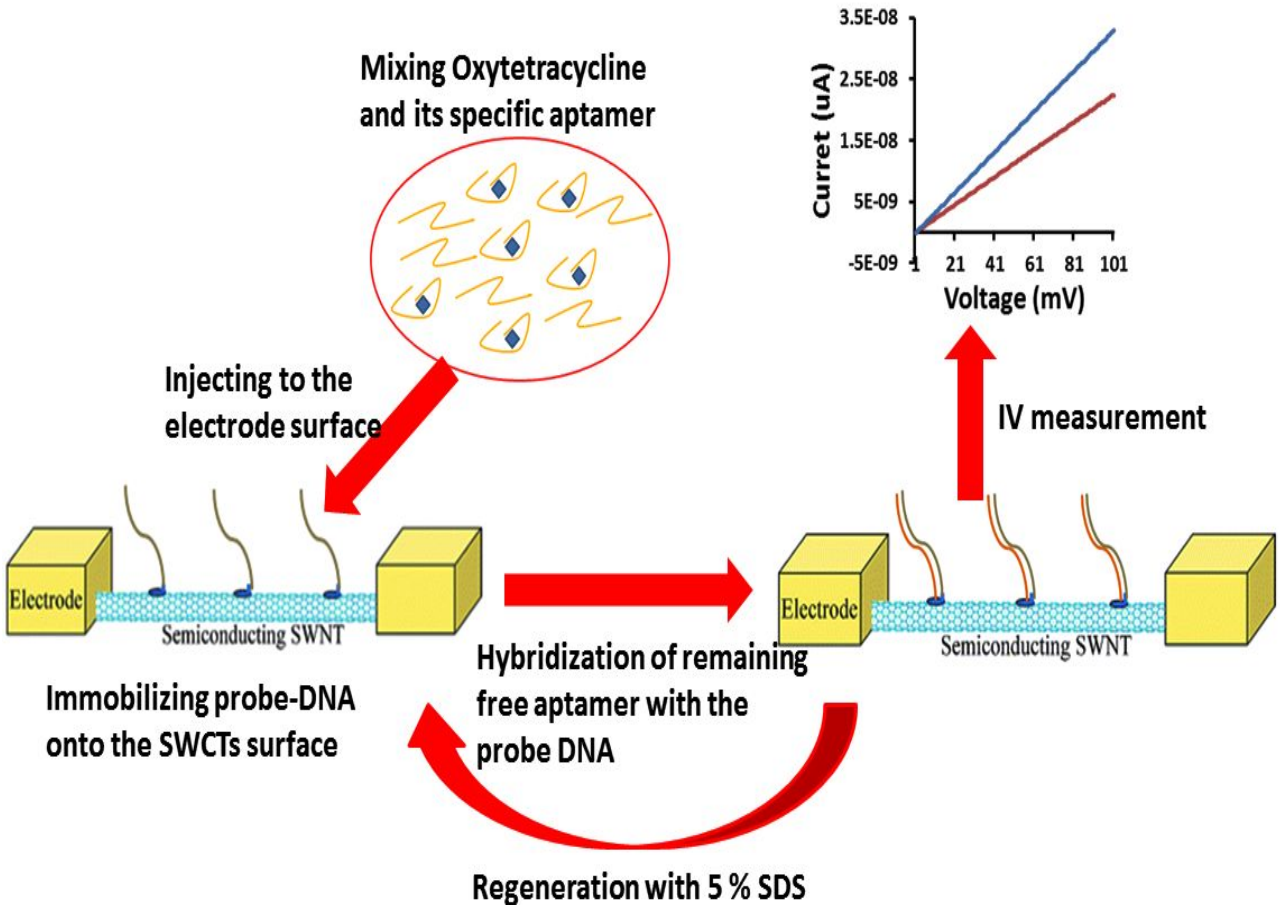

Figure 1. Schematic representation of sensing mechanism for OTC (oxytetracycline) detection using aptamer-based SWCNTs biosensor. The sensing mechanism and procedure involves pre-mixing and incubation of water sample with known concentration of aptamer, hybridization of residual free OTC-aptamers with probe-DNA immobilized on SWCNTs surface, conductivity change detection and regeneration steps.

Probe-DNA Immobilization. Probe DNA was immobilized onto SWCNTs bridge using non-covalent bonding (π-π stacking interaction between the pyrene group and the SWCNTs surface). PBSE was utilized as a linker between probe-DNA and SWCNTs. Immobilization of aminated probe DNA (NH_2-DNA) onto PBSE-SWCNTs was performed using a covalent bond

(figure S2). When SWCNTs bridge was modified with linker and probe DNA, current responses (μA) were decreased to ca. 0.03 and ca. 0.02, respectively (Fig. 2).

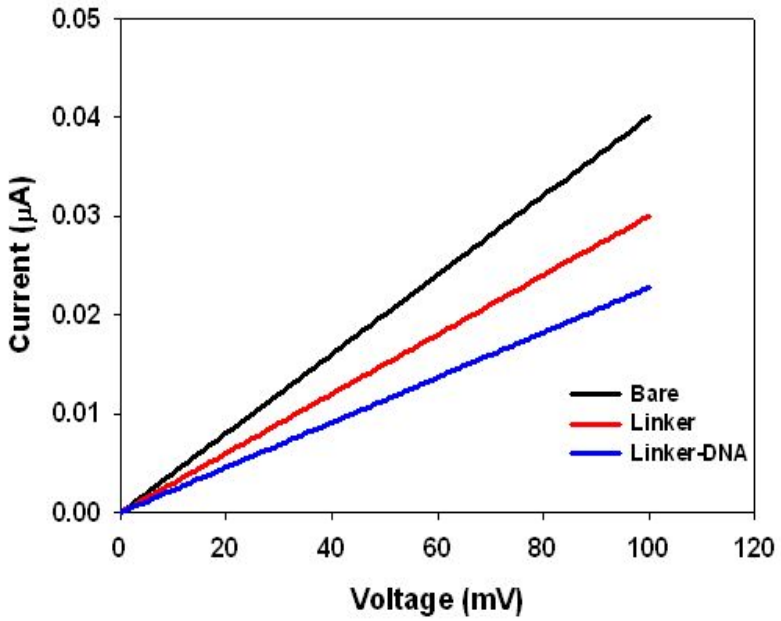

Figure 2. IV measurement responses of SWCNTs based system for bare electrode, linker and probe-DNA immobilized electrode.

The resistance changes in the device are dependent the number of contacts of the elements within the SWCNTs network. Molecular interactions disrupt the network continuity resulting in increased resistance. Percolation phenomena are determined by the concentrations of conductive materials in the system. Therefore, high concentration of SWCNTs leads to good current flow while the modified SWCNTs inhibits the percolation and results in the increased resistance [49].

Optimization of the Sensing Conditions. Different aptamer concentrations (0.1, 0.5, 1.0, 10, 50, and 100 μg/L) in the pre-mixing step were performed to determine the optimal aptamer concentration. Varying probe-DNA concentrations at 0.01 mg/ml and 0.05 mg/ml were used at

immobilization step to determine the convenient one. For each aptamer concentration, the I-V profiles were observed before and after aptamer injection to the chip surface and relative resistance differences ($\Delta R/R_0$) were calculated.

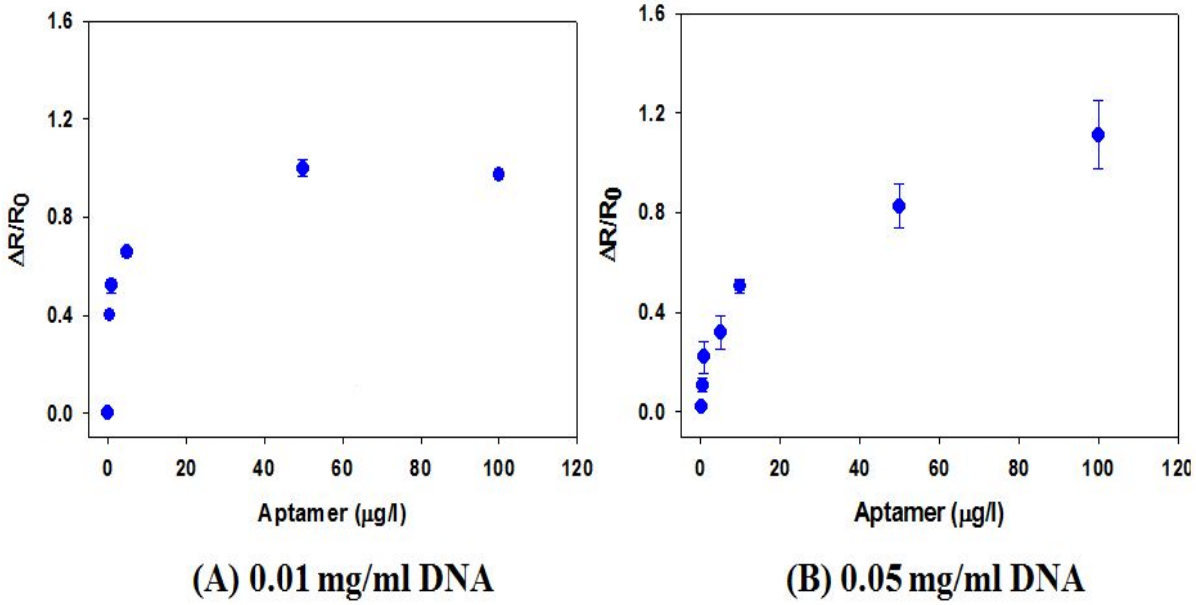

Figure 3. Calibration of aptamer concentrations (0.1, 0.5, 1.0, 10, 50, and 100 μg/L) using R responses. A) 0.01 mg/ml probe-DNA B) 0.05 mg/ml probe-DNA immobilization.

As shown in Figure 3, 0.5 mg/ml probe-DNA exhibited wider linear range and higher signal (more differences in the $\Delta R/R_0$). Thus, we selected the 0.05 mg/ml probe-DNA concentration for the following experiments. 100 μg/ml aptamer concentration was selected for further experiments because this concentration led to the highest $\Delta R/R_0$ value and was near the plateau range for the surface binding.

Several incubation time lengths (1, 3, 6 and 10 min) for the pre-mixing of environmental relevant OTC concentration (75 μg/L) and OTC-aptamer (100 μg/L) were evaluated (Figure S4). Prolonged incubation time of the OTC with aptamer led to decrease in the sensor signal but approaching a

plateau level after 6 min. Therefore, we chose to use 6 min of incubation time for all the subsequent analysis.

Dose-response Measurements and detection limit. Different concentration of OTC (0 ,10, 25, 50, 75, 100, 150, and 200 μg/L) and 100 μg/L OTC-aptamer were mixed for 6 minutes (as optimized before) and injected to the gold chip surface. Before this injection, the background I-V profile was observed for the gold electrode. After hybridization to allow the free aptamers to bind with the probe-DNA immobilized onto the SWCNTs surface, the I-V profile of the electrode was measured again. The relative resistance differences ($\Delta R/R_0$) were calculated depend on the initial resistance values for each OTC concentrations.

The increase in the OTC concentrations in the sample, after pre-incubation with known aptamer concentration, led to proportional decrease in residual free aptamer, therefore the decrease in $\Delta R/R_0$ d. Figure 4 shows the calibration curve for OTC, the error bars in the figure correspond to the standard deviations of the data points in five independent experiments, with the coefficient of variation of all the data points being within 3-21%.

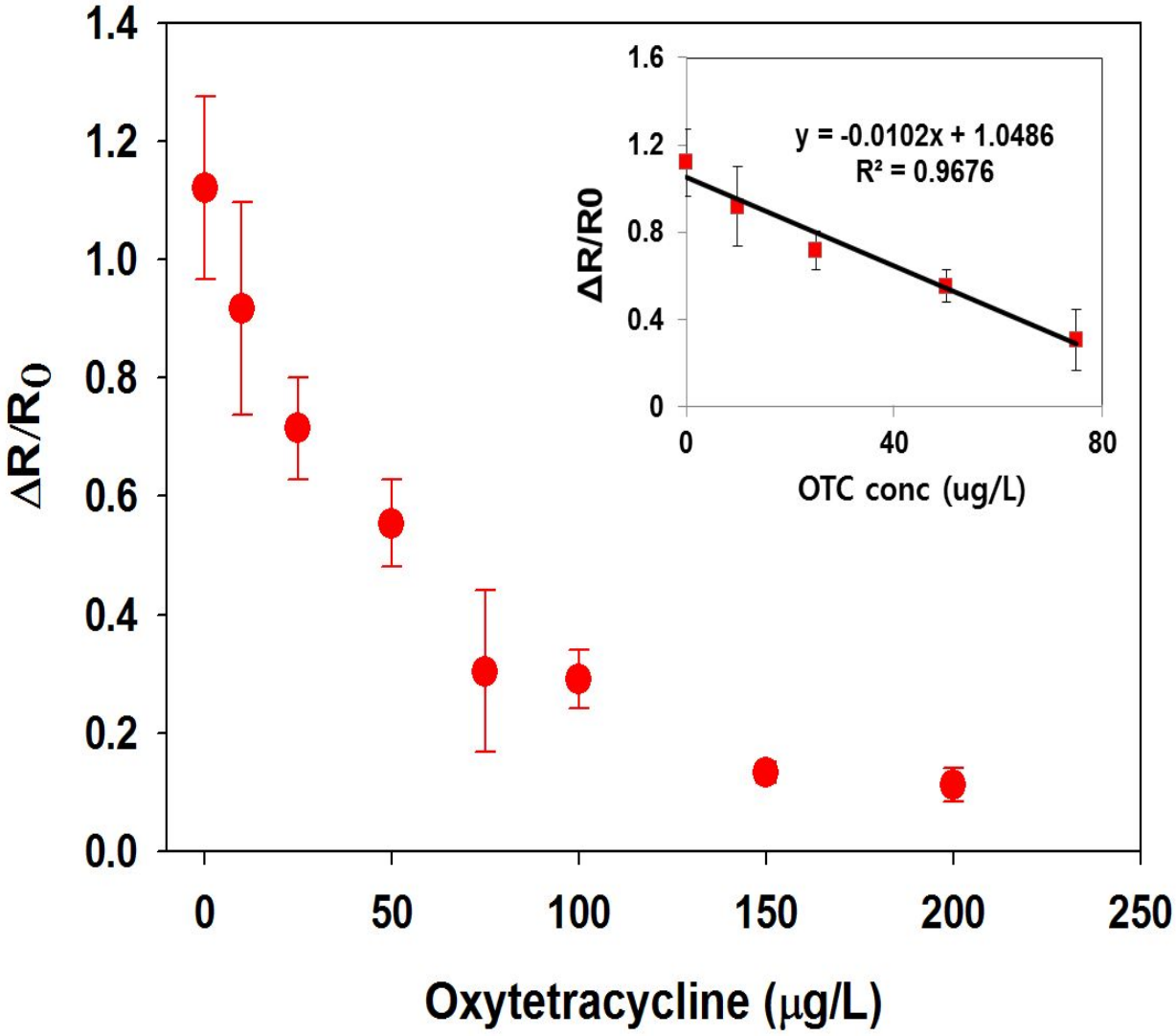

Figure 4. Dose response characteristics of aptamer based SWCNTs biosensors for Oxytetracycline detection (OTC concentrations at 0, 10, 25, 50, 75, 100, 150, and 200 μg/L). Linear detection range of OTC (inner figure). Each data value is the average of five independent experimental results.

The linear range was between 10 and 75μg/L (20~325 nM) and a detection limit (LOD) of 1.125 μg/L (2.5 nM) was derived according to Armbruster et. all. [50], where the LOD is determined based on the dose response curves as 3 times of the signal standard deviation, where the actual tested

LOD is 10 μg/L (~20 nM). The detection limit we obtained is comparable to those reported in the literature as summarized in Table 1. In addition, compared to other sensors mentioned in table 1, the biosensor developed here is simpler and faster (less than 15 minutes, including pre- incubation, measurement and regeneration). And the miniature and portable platform also allows for potential on-site or real-time measurements.

Specificity Evaluation of the SWCNT biosensor. To determine the specificity of the aptamer biosensor for detecting OTC, different antibiotics such as amoxicillin, diaminofen, genomiycin, amphotericin and ciprofloxacin were evaluated. The biosensor system's responses for these chemicals were compared with the results of OTC detection and control experiment (100 μg/L OTC-aptamer without any antibiotics).

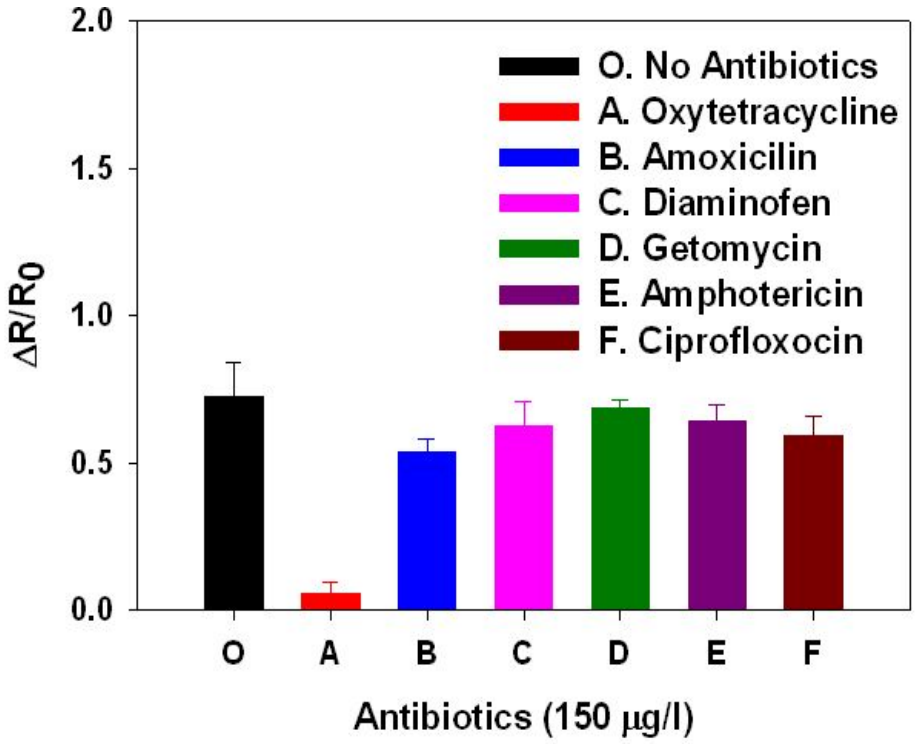

Figure 5. SWCNT aptamer biosensor specificity assessment via comparison of sensor signals of OTC, with other antibiotics. All chemicals are tested at 150 μg/L level (concentration represent

the higher range reported in literature), and each data value is the average of three independent experimental results.

The results in Figure 5 clearly show that the signals of various antibiotics tested were comparable to the blank control with no antibiotics, whereas the signal for OTC is less than 20 % of control experiment. Therefore, the specificity of the developed biosensor is acceptable for this kind of small molecule detection systems [51].

Regeneration, Reusability and Sensor Stability. The regeneration performance of the sensing systems is important for practical implementation of biosensors [52]. Therefore, in the present system, the reusability and stability were evaluated. 5 independent experiments were performed for 5 different OTC concentrations (10, 25, 50, 100 and 150 µg/L) and the $\Delta R/R_0$ responses were calculated for each analysis. With less than 15 % of signal reduction, the regeneration step was working quite well for the developed system (figure 6A). For the 5 independent experiments sd and cv % values were observed around 0.005-0.016 and 2.2 %-10.3 %. The storage stability of the system was evaluated by performing three daily measurements over 30 days of continuous analysis (figure 6B) and the reduction of the % response (calculated depend on the first day result) was less than 10 % (In the inside figure of figure 6B) with the 0.04-3.6 of sd and 6.1%-13.8% of cv % values. This slight drop in resistance signal did not affect the specific response of these kinds of biosensor systems. Depends on the results, the sensor offers comparable or higher reusability and stability compared to other antibiotics detection system have been reported (table 1).

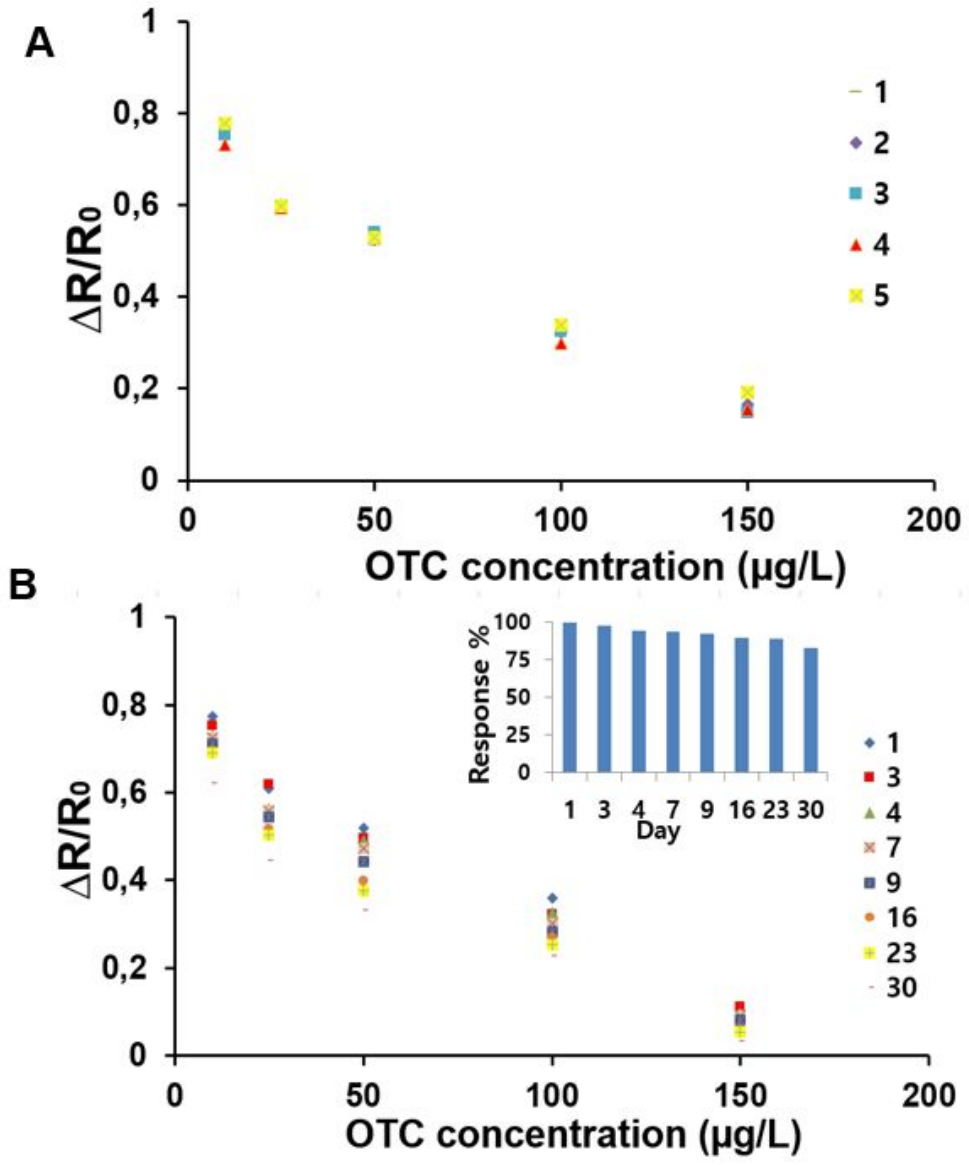

Figure 6. A. Assessment of the reusability of the OTC sensing system depend on the $\Delta R/R_0$ responses for 5 different OTC concentrations. Signals for daily average of three measurements of 5 different concentrations of OTC.**B.** Assessment of the stability of the OTC sensing system depend on the $\Delta R/R_0$ responses measured at 8 different days for the same OTC concentrations. In the inside figure B, % of responses values were calculated compared in relevance to the value on the first day.

Analysis of Spiked environmental water Samples. To evaluate the potential matrix effect of real environmental water sample on the sensor performance, we analyzed spiked samples that contained different concentrations of OTC (10 and 75 µg/L) in tap water and two different wastewater effluents from different wastewater treatment plants in US. The results were summarized in Table 2. The recovery of all measured samples was between 86.4 and 95.8 %, and the parallel tests showed that the relativity coefficient was within 3.2 - 12.8 %, (n = 3). These results indicated that the possible interference from the different background composition of waste water effluent and tap water samples was within 3.2- 12.8% and is considered acceptable for environmental applications [53-55].

Table 2. Detection results of OTC-spiked wastewater samples

	Spiked µg/L	Found µg/L	cv %	recovery %
plant 1	10	9.57	3.2	95.8
	75	69.94	10.2	92.3
plant 1	10	9.35	4.2	93.5
	75	71.40	12.8	95.2
tap water	10	8.63	5.3	86.4
	75	71.13	9.0	94.8

In conclusion, a simple and highly sensitive aptamer-based single walled carbon nanotubes (SWCNTs) biosensor containing probe-DNA immobilized on functionalized SWCNTs was

developed for a miniature environmental monitoring with faster response time. In addition, due to its inherent small size and the relatively easy and cost-effective fabrications process, a compact system with multiple (up to tens or hundreds) sensors can be used for small portable model application. These aspects make our system superior compare to the FETs (field effect transistors) systems for sensitive, rapid and cost effective environmental pollutants detection. Results showed that the developed biosensor is able to detect oxytetracycline in the range between 20~325 nM that sufficiently cover the range of OTC concentrations detected in the environment. Compared with our developed sensing systems reported in the literature (Table 1), the detection range and detection limit are comparable and more sensitive than most of them. The specific advantages and features of this newly developed SWCNT sensor include fast response, relatively comparable or lower detection limit, reusability (over 20 times) and stability up to 30 days with less than <15% signal decrease. Furthermore, the biosensor developed here could be readily extended toward the on-site monitoring of the other trace small molecular pollutants in environmental matrices with the employment of different probes modified by other analyte conjugates and specific aptamers. Additionally, our novel, easy and cost-effective fabrications process for SWCNT assembly enables possible multi-sensor array systems that cannot be easily done with other sensors reported.

ASSOCIATED CONTENT

Supporting Information;

Detailed descriptions of the flexible biosensor processes and SEM images (Figure S1) and immobilization procedure of probe-molecules (complementary DNA molecule to the OTC aptamer sequence) onto SWCNTs bridge surface (Figure S2 and S3) and pre-mixing time length optimization (Figure S4) results are available in the supporting materials.

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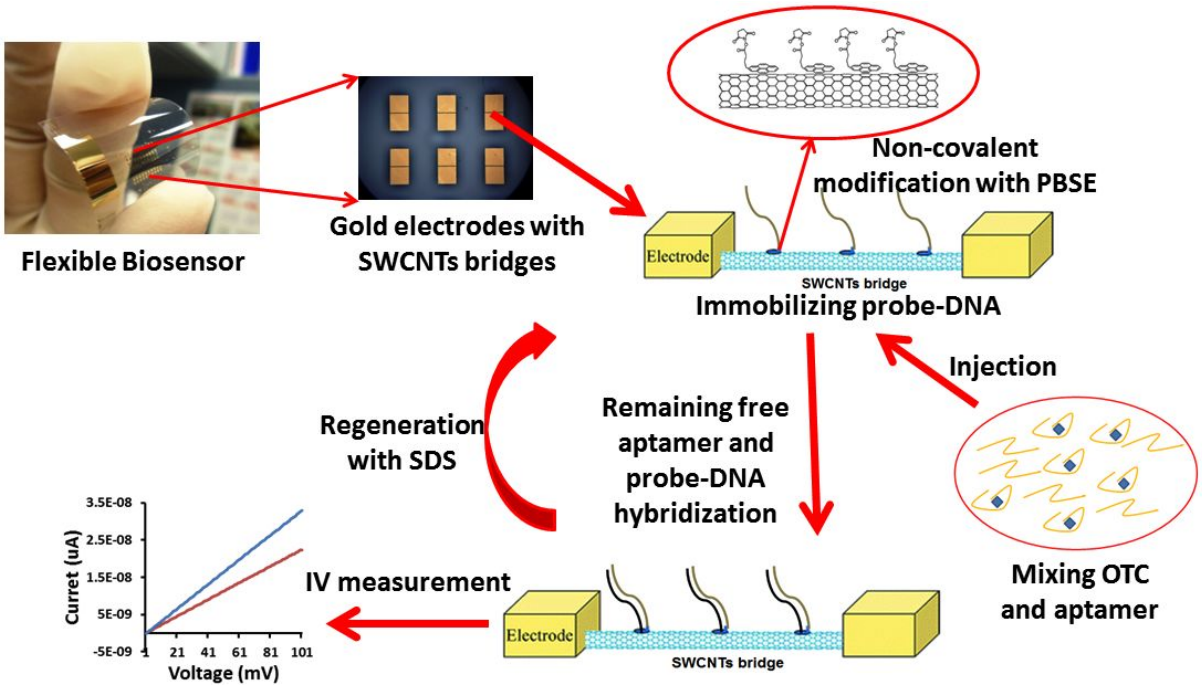
Flexible Biosensor
Gold electrodes with SWCNTs bridges
Non-covalent modification with PBSE
Electrode
SWCNTs bridge
Immobilizing probe-DNA
Injection
Mixing OTC and aptamer
Remaining free aptamer and probe-DNA hybridization
Regeneration with SDS
IV measurement
Curret (uA)
Voltage (mV)
3.5E-08
2.5E-08
1.5E-08
5E-09
-5E-09
1
21
41
61
81
101