Development of Carbon Nanotube-based Biosensor for Monitoring Microcystin-LR in water

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1. A completion report

The occurrence of blue-green algae, a.k.a. cyanobacterial blooms, has increased in freshwater lakes, basins, rivers and ponds throughout the world including America [1, 2], Argentina [3], Australia [4], Spain [5], Uganda [6], and China [7] due to the eutrophication and warm temperature of surface water. In the summer of 2010, blue-green algae blooms with excessive concentration of microcystin toxin occurred and warnings were issued in 20 public lakes and ponds across the state of Ohio, including Grand Lake St. Mary, Ohio's largest inland lake. The level of microcystins (MCs) ranged from 5.4 μg/L to 67.3 μg/L in several freshwater reservoirs and inland lakes in Ohio [8]. In addition, in November, 2010, the USEPA issued new standards for protection of Florida waters in order to keep clean water safe from HABs pollution [9]. In California, the death of sea otters by microcystin toxin from freshwater was reported in September, 2010 [10]. Australia also issued a guideline to protect public health from the threat of toxins, which were released from blue-green algae in 2010 [11]. In recent research, Graham et al. obtained samples from cyanobacterial blooms in 23 Midwestern United State lakes to evaluate potential human health risks and reported that 96% of blooms contained toxin-producing cyanobacteria such as *Aphanizomenon*, *Cylindrospermopsis*, and *Microcystis*. In particular, microcystins, which are deleterious hepatotoxins, were found in all blooms [1]. Among the hepatotoxins, MCs are the most commonly reported cyanotoxins and MC-LR is the most frequently occurring variant among MCs in the United States and throughout the world [12-14]. The LD_{50} values of MC-LR for mouse bioassay and brine shrimp assay are 25-150 μg/kg and 5-10 mg/L, respectively [15]. The provisional concentration limit of MC-LR in drinking water of 1 μg/L was assigned by the World Health Organization (WHO) [16]. Current methods to detect MC-LR in various matrices require long processing times, sophisticated instruments, complex procedures and high expenses. A sensitive, specific, and simple method for monitoring MCs is necessary to immediately institute remedial measures to prevent exposure to these toxins. To this end, carbon nanotubes' electrical and physical properties (e.g. high electrical conductivity, huge electrochemically active surface area, and broad working area) are desirable for developing novel submicron sized electrochemical sensors. An innovative, field-portable, continuously monitoring multi-walled carbon nanotube (MWCNT)-based biosensor can detect MC-LR at low detection limits. During this period of the project (May 1, 2011 through April 30, 2012), various research activities were conducted to develop MWCNT-based biosensor for monitoring of a cyanobacterial toxin, MC-LR.

1.1 Research Objectives

The physical and electrochemical properties (e.g. high electrical conductivity, ease of functionalization, huge electrochemically active surface area, and broad working area) of MWCNTs make them a candidate material for the development of electrochemical biosensors/immunosensors. Electrochemical biosensors using antibodies are also very promising for on-site monitoring of MC-LR due to the high selectivity, low expenses, and simplicity of the detection method. The objectives of the project were to investigate the methods/procedures to fabricate MWCNT-based electrode and functionalize the interface of MWCNT for sensing applications and investigate the method of integration between monoclonal antibodies/protein phosphatases and MWCNT to achieve specificity of toxin identification as well as to develop, characterize, and evaluate sensor performance for identification of MC-LR.

1.2 Methodology and Findings

In this study, we reported three main findings. Firstly, dense arrays of MWCNT were synthesized in controlled shapes by using a patterned catalyst with water-assisted chemical vapor deposition (CVD). The MWCNT-based electrodes were fabricated with a grown MWCNT array. The electrodes were functionalized with potentiostatic treatment in alkaline solution in order to produce polar groups on the interface of MWCNT array for the cross-linking reaction with cross-linking agents and immobilization of biomolecules. The electrochemical properties of MWCNT electrodes were characterized with cyclic voltammetry, micro-Raman spectroscopy, X-ray photoelectron spectroscopy (XPS), optical microscopy, and electrochemical impedance spectroscopy (EIS) before and after functionalization. Cyclic voltammetry of fabricated electrodes shows well-defined redox peaks in the presence of redox species before and after functionalization, which indicates MWCNTs are a suitable candidate as a sensing material. From the micro-Raman analysis, the higher the intensity ratio of the D to the G-band the more functional groups (i.e. defects in the graphitic structure or even amorphous carbon) are formed on the MWCNTs after functionalization, thus indicating that the functionalization process is indeed effective. In addition, the increase of oxygen species and ratio of a C-C:C=O after the functionalization in XPS analysis corroborated the micro-Raman analysis for the functionalization. Secondly, the MWCNT-based biosensors were prepared by immobilization of MC-LR onto the functionalized MWCNT array electrodes. The change of electron-transfer resistance of prepared biosensors following the conjugation process using MC-LR and antibody was measured with EIS in order to show the efficient sensing properties of the MWCNT-based biosensor as electrochemical biosensors. The increase of the electron-transfer resistance with the process of MC-LR and antibody conjugation through the electrochemical impedance spectroscopy study indicated the proposed technique is very useful for monitoring of

MC-LR. Finally, the calibration curve was established by using the change of electron-transfer resistance using different concentration of MC-LR. The difference of electron-transfer resistance was greater at lower concentration of MC-LR. The working range of MWCNT based biosensor was from 0.05 to 20 $\mu g \cdot L^{-1}$. The WHO provisional concentration limit (1 $\mu g \cdot L^{-1}$) [16] is much higher than our calculated method detection limit of 0.04 $\mu g \cdot L^{-1}$.

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2. Publication citations

I. Journal Articles

(1) Amos Doepke, Changseok Han, Tyson Back, Wondong Cho, Dionysios D. Dionysiou, Vesselin Shanov, H. Brian Halsall, William R. Heineman, Analysis of the electrochemical oxidation of multi-walled carbon nanotube tower electrodes in sodium hydroxide, *Accepted to Electroanalysis.*

(2) Changseok Han, Amos Doepke, Wondong Cho, Vlassis Likodimos, Armah A. de la Cruz, Tyson Back, William R. Heineman, H. Brian Halsall, Vesselin N. Shanov, Mark J. Schulz, Polycarpos Falaras and Dionysios D. Dionysiou, Multi-Walled Carbon Nanotubes-based Biosensor for Monitoring Microcystin-LR in Drinking Water Sources, *In preparation for submission, May 2012*.

II. Presentations

(1) Changseok Han*, Amos Doepke, Wondong Cho, Armah A. de la Cruz, William R. Heineman, H. Brian Halsall, Vesselin N. Shanov, Mark J. Schulz, Vlassis Likodimos, Polycarpos Falaras, and Dionysios D. Dionysiou, Multi-walled Carbon Nanotubes-based Biosensor for Monitoring Cyanotoxins in Drinking Water Sources. Oral Presentation at the 243rd American Chemical Society (ACS) National Meeting, Symposium on Environmental Applications and Ecological Implications of Nanotubes, Nanowires, and Fullerenes, Division of Environmental Chemistry, paper 528, March 25-29, 2012, San Diego, California.

(2) Changseok Han*, Amos Doepke, Wondong Cho, Armah A. de la Cruz, William R. Heineman, H. Brian Halsall, Vesselin N. Shanov, Mark J. Schulz, Vlassis Likodimos, Polycarpos Falaras and Dionysios D. Dionysiou, Carbon Nanotubes-based Biosensor for Detecting Cyanotoxins in Water. Poster Presentation at the 3rd International Conference from Nanoparticles and Nanomaterials to Nanodevices and Nanosystems (IC4N-3), June 26-30, 2011, Crete Island, Greece.

3. Students supported by the project

(1) Changseok Han
Ph.D. student, Environmental Engineering;

4. Brief description of notable awards or achievements resulting from the project

(1) 2012 *Certificate of Merit Award for First Paper Presentation*, American Chemical Society, Division of Environmental Chemistry, ACS 243rd meeting, March 25-29, San Diego.

(2) 2011 Best Poster Paper Award, 3rd International Conference from Nanoparticles and Nanomaterials to Nanodevices and Nanosystems, June 26-30, Crete, Greece.

(3) 2011 National Science Foundation Travel Award to attend the 3rd International Conference from Nanoparticles and Nanomaterials to Nanodevices and Nanosystems and the NSF Workshop; Participation in the Poster Competition, June 26-30, Crete, Greece.

5. Appendix List.

Appendix A-accepted paper. Analysis of the electrochemical oxidation of multi-walled carbon nanotube tower electrodes in sodium hydroxide.