A label-free impedance immunosensor based on multi-walled carbon nanotube electrode for rapid and sensitive detection of *E. coli* O157:H7

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Abstract

- Outbreaks of foodborne diseases have drawn great public attention globally and the key to ensure food safety is rapid detection of pathogenic bacteria. Traditional detection methods, such as bacterial culture, polymerase chain reaction and enzyme-linked immune-sorbent assay are limited by either complex sample preparation, inefficiency in time or sensitivity.
- Nanomaterials have been studied to enhance biosensors for better performance. Our multi-walled carbon nanotube (MWCNT) with high surface-to-volume ratio has shown improved efficiency in biomolecule immobilization and signal generation.
- In this study, we developed a label-free impedance immunosensor based on a homemade MWCNT electrode for rapid and sensitive detection of *E. coli* O157:H7. First, the MWCNT electrode was fabricated by "drop-cast" of carbon nanotubes on a gold surface. N-hydroxysuccinimide/1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (NHS/EDC) was used to activate the carboxyl groups on MWCNTs and streptavidin was then covalently bonded. Biotin-*E. coli* O157:H7 antibodies were immobilized on the MWCNTs through biotin-streptavidin conjugation. 1% BSA was used to block the non-specific binding sites on the MWCNTs.
- For testing, 50 µl sample containing *E. coli* O157:H7 cells was dropped on the surface of the MWCNT electrode. The target pathogens were then captured by the antibodies, resulting in impedance change correlated to the concentration of bacterial cells. The results showed that the proposed immunosensor was capable of detecting *E. coli* O157:H7 in a concentration range from 10^2 to 10^8 CFU/ml. The detection chamber was designed and fabricated using a 3D printer. The total detection time from sampling to signal acquisition was less than 1 h.
- This immunosensor could be integrated with a laptop-based data acquisition and processing as a portable device for on-line or in-field use. Currently, we are working on the optimization of parameters for the fabrication of the MWCNT electrode and the testing of different food samples to validate the immunosensor for its application in food supply chain.

Introduction

- The US Department of Agriculture (USDA) estimates that foodborne illnesses cost $15.6 billion each year. Almost 1 in 10 people in the world fall ill after consuming contaminated food and 420 000 die every year. Among all the foodborne diseases, bacteria and viruses are the most common causes of food poisoning.
- *E. coli* O157:H7 is one of the most common foodborne pathogens that cause illnesses, hospitalizations, and deaths in the United States. As of May 15, 2018, 172 people infected with *E. coli* O157:H7, including one death, have been reported from 32 states.
- It is highly important to develop a fast, reliable and cost-effective foodborne pathogen detection method for *E. coli* O157:H7 to ensure food safety.

Objective

The objective of this study is to develop a MWCNT based immunosensor for rapid, sensitive detection of *E. coli* O157:H7 .

Materials & Methods

- **Bacteria**: *E. coli* O157:H7 (ATCC 43888); *Salmonella* Typhimurium (ATCC 14028); *E. coli* K12 (ATCC 29425); *Listeria monocytogenes* (ATCC 43251); *Listeria innocua* (ATCC 33090)
- **Reagents**: NHS/EDC, streptavidin, biotin labeled rabbit anti-*E. coli* O157:H7 antibodies, bovine serum albumin (BSA), ethanol, synthesized MWCNTs (around 16 nm in diameter).

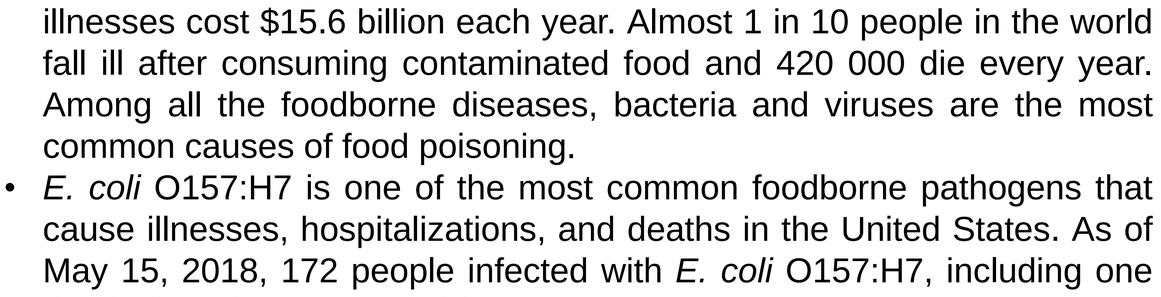

Fig. 1 (a) Detection chamber design (b) "Drop-cast" electrode (c) MWCNT electrode in the flow cell

Materials & Methods (Cont'd)

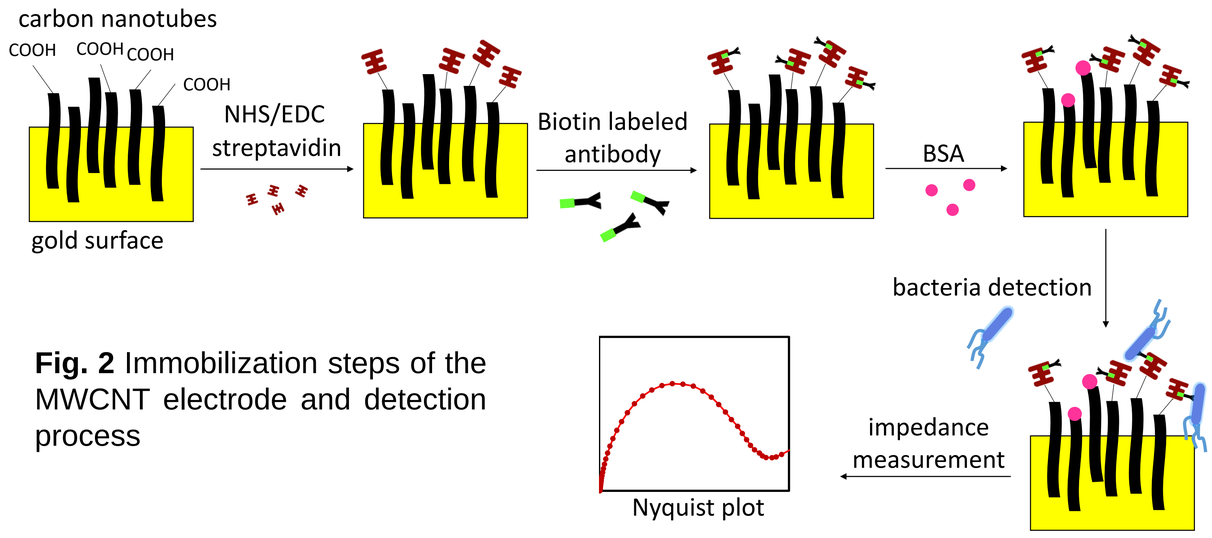

Fig. 2 Immobilization steps of the MWCNT electrode and detection process

Results

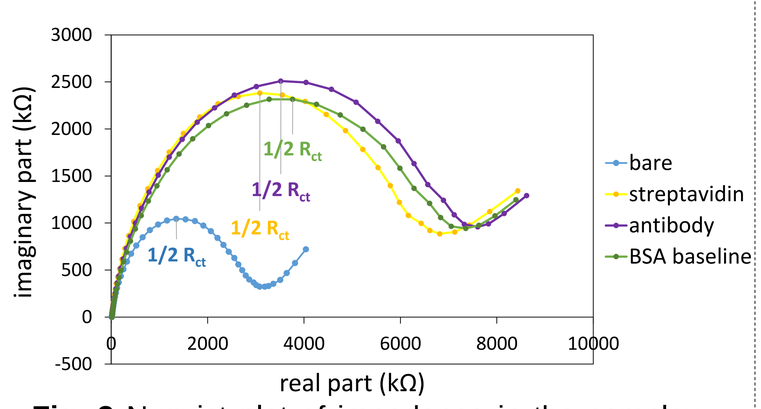

Fig. 3 Nyquist plot of impedance in the complex plane of immobilization steps (R_{ct} stands for resistance of charge transfer)

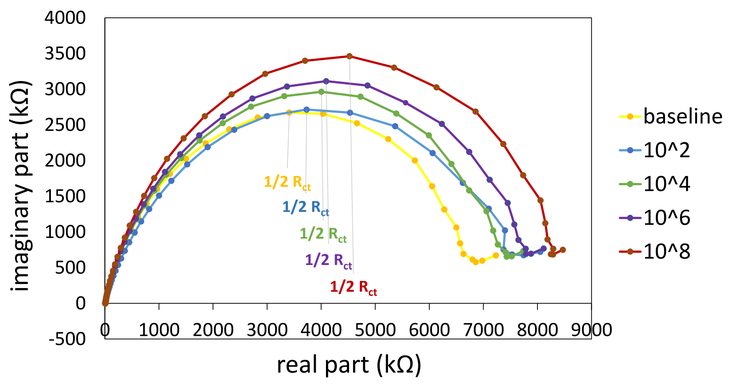

Fig. 4 Nyquist plot of impedance in the complex plane with different concentrations of *E. coli* O157:H7 (CFU/ml)

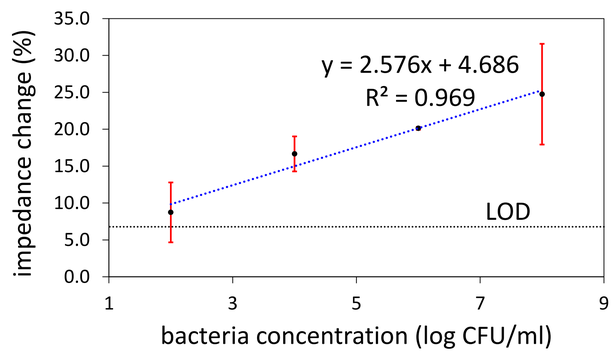

Fig. 7 Linear regression of R_{ct} change (%) with the concentration of *E. coli* O157:H7

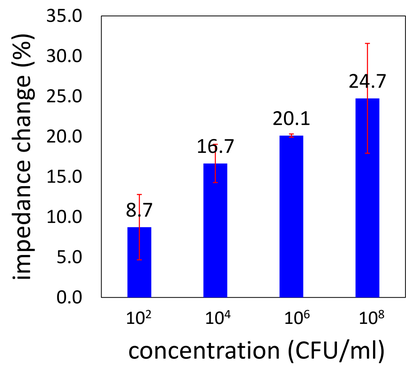

Fig. 5 R_{ct} change (%, the percentage of R_{ct} change compared to the R_{ct} of baseline) in sensitivity test using *E. coli* O157:H7. The positive response indicates successful binding and detection of target

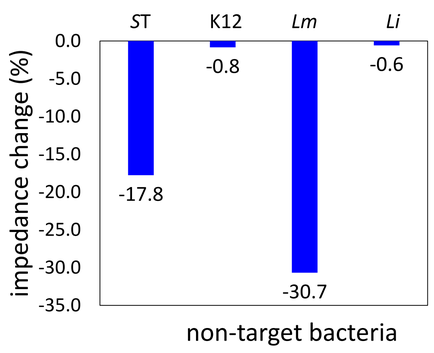

Fig. 6 R_{ct} change (%) in non-specific test using 10^5 CFU/ml *Salmonella* Typhimurium, *E. coli* K12, *Listeria monocytogenes* and *Listeria innocua.* The negative response indicates negligible binding of non-target bacteria and good specificity

Conclusions

- A MWCNT-based impedance immunosensor was developed for detection of *E. coli* O157:H7.
- The results showed that the proposed immunosensor could detect as low as 10^2 CFU/ml bacteria cells in less than 1 h, and no positive signal from non-target bacteria was observed, which suggested good specificity of the immunosensor.
- The flow cell that the immunosensor is based on can be connected to injection pumps and can be easily automated.

Acknowledgements

This research was funded by Walmart Foundation and supported by Walmart Food Safety Collaboration Center (002162-00001A) and Arkansas Bioscience Institute (0383 43076-24-2333). The authors thank Dr. Ping Yao for her help with the 3D printing of the detection chamber.

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