Rapid Detection of *Salmonella* Typhimurium in Poultry Using a Portable Immunosensing System

America Sotero[1], Xinge Xi[1], Tao Wen[1, 3], Ronghui Wang [1], Yanbin Li [1, 2]

[1]Department of Biological and Agricultural Engineering, [2]Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, AR 72701, USA
[3]School of Mechanical and Electrical Engineering, Central South University of Forestry and Technology, Changsha, Hunan 410004, China

Abstract

A portable impedimetric immunosensing system for the rapid and sensitive detection of *Salmonella* Typhimurium in poultry was developed using an interdigitated microarray electrode (IDAM). The immunosensing system consisted of a gold IDAM, a signal acquisitive interface, and a laptop computer with LabVIEW software. The IDAM was first functionalized with 20mM 16-Mercaptohexadecanoic acid followed by surface immobilization with streptavidin and biotin-labeled *S.* Typhimurium-antibody. Serially diluted samples of *S.* Typhimurium were dropped onto the surface of the IDAM, which allowed the immobilized antibodies to capture the *Salmonella* cells. The capture of the cells resulted in impedance changes which were measured and displayed using the laptop with LabVIEW software. There was a linear relationship with a correlation coefficient of 0.98 between the impedance change and the log value of *S.* Typhimurium in concentrations of 7.6×10^1 to 7.6×10^6 CFU (50 µl)$^{-1}$ when pure culture samples were dropped onto the surface of the IDAM. A flow cell imbedded with an IDAM was also used to detect *Salmonella* Typhimurium in contaminated chicken rinse water and had correlation coefficient of 0.66. The limit of detection (LOD) of *S.* Typhimurium in contaminated chicken rinse water and pure culture samples was 7.6×10^2 CFU (50 µl)$^{-1}$. The detection time from the moment a sample was dropped onto the IDAM surface to the display of the results on the laptop was 1 hr. The developed portable impedance immunosensor shortens detection time and has a limit of detection (LOD) that is comparable to commercial impedance instruments. The use of the imbedded IDAM flow cell can also reduce the potential for contamination due to the nature of the close system. Due to its faster detection time compared to traditional methods, low cost, label-free feature, and portability the developed immunosensor has the potential to improve in-field detection of foodborne pathogens..

Introduction

Each year, *Salmonella* Typhimurium causes an estimated 19,000 hospitalizations and 380 deaths [1]. It is considered one of the most dangerous foodborne pathogens and a major threat to human health. *S.* Typhimurium is typically transmitted to people through the consumption of food products such as poultry, meat, eggs and milk [2] . Traditional methods that depend on microbiological methods for detection of *Salmonella* are time consuming and labor intensive since they require multiple steps for enrichment and growth of the bacteria [3]. Newer methods using impedimetric biosensors for detection of foodborne pathogens also have several limitations. These limitations include low efficiency, lack of pathogen specificity, and lack of portability for in-field detection. Therefore, there is an urgent need for the development of a rapid and reliable method to detect *Salmonella* in food products. The goal of this project was to develop a portable impedance immunosensing system using an interdigitated microarray electrode (IDAM) for the rapid and sensitive detection of *S.* Typhimurium in poultry products.

Objectives

1. To develop a portable impedimetric immunosensing system:
   - For rapid detection of *S.* Typhimurium in poultry
   - With high specificity for *S.* Typhimurium
   - Comparable with commercial electrochemical impedance instruments
2. Simulate the behavior of the biosensor using an equivalent circuit

Materials and Methods

Bacteria

- *E. coli* O157:H7, ATCC 43888 *E. coli* K12
- *L. innocua* *L. monocytogenes*, ATCC 43251

Reagents

- 16-Mercaptohexadecanoic acid 20mM (MHDA)
- EDC/NHS 75mM/30mM, v/v 1:1
- Streptavidin 50µL, 1 mg/mL
- Biotin labeled S. Typhimurium antibodies, 4.0-5.0 mg ml-1
- 50 µl of BSA (1% in PBS)
- $[Fe(CN)_6]^{3-/4-}$ as redox probe

Principle of Immunosensor

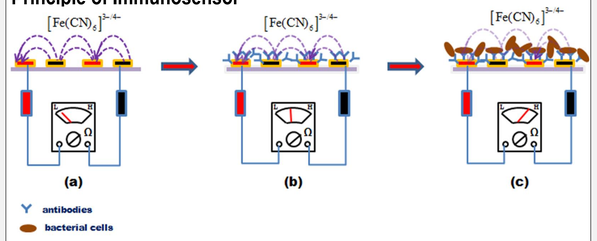

Fig. 1. Principle of the immunosensor: (a) bare IDAM; (b) IDAM with antibody immobilization; and (c) IDAM with bind bacterial cells

Immunosensing system setup

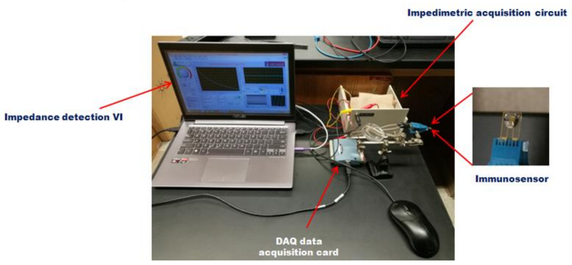

Fig. 2. Setup of the portable immunosensing system

Immunosensing system with flow cell setup

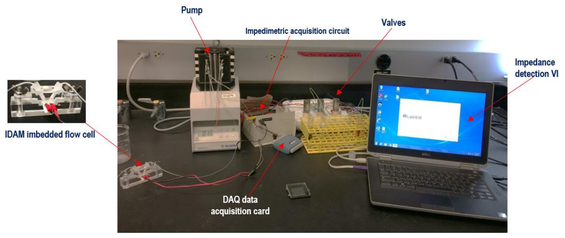

Fig. 3. Setup of portable immunosensing system with IDAM imbedded flow cell

Equivalent circuit of immunosensor

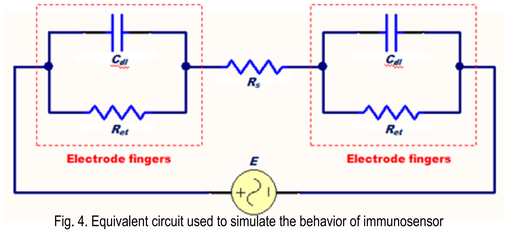

Fig. 4. Equivalent circuit used to simulate the behavior of immunosensor

Flowchart of assay

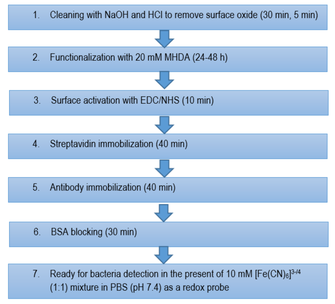

Fig. 5. Flowchart of the assay

Results and Discussion

Capture of *S.* Typhimurium cells on IDAM surface

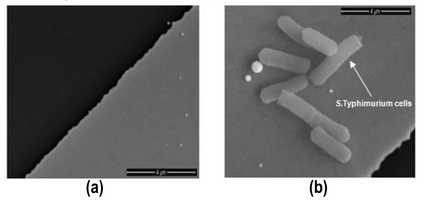

Fig. 6. (a) SEM images of antibodies immobilized on IDAM (b) S. Typhimurium cells captured by immobilized antibodies

S. Typhimurium impedance detection

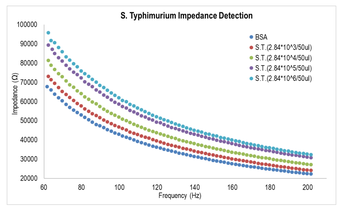

Fig. 7. Recorded impedance detection results for pure culture samples dropped onto IDAM surface

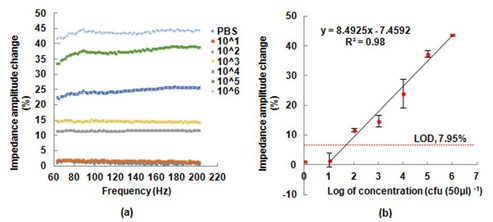

Fig. 8. (a) Impedance change of negative control (PBS) and concentrations of S. Typhimurium in pure culture, 10^1 to10^6 CFU (50 µl)$^{-1}$ and (b) relationship between logarithmic value of concentration and impedance change at 101 Hz.

Table 1. Paired t-test samples of negative control and different concentrations of S. Typhimurium in pure culture samples

Paired Samples	Mean Difference (%)	Std. (%)	P-values
NC - 10^1	-0.2	0.41	0.053
10^1-10^2	-10.21	0.26	<0.01
10^2-10^3	-2.8	0.24	<0.01
10^3-10^4	-10.10	1.01	<0.01
10^4-10^5	-13.09	0.47	<0.01
10^5-10^6	-6.12	0.66	<0.01

Specificity of immunosensor

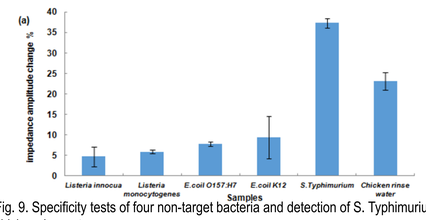

Fig. 9. Specificity tests of four non-target bacteria and detection of S. Typhimurium in chicken rise water

Conclusions

The equivalent circuit showed that the greatest change in impedance was found in the electron-transfer resistance. The change in impedance can be attributed to the barrier formed when *S.* Typhimurium cells bind to the antibody-immobilized electrode surface. The immunosensor had a high specificity for detection of S. Typhimurium cells with a LOD of 10^2 CFU (50 µl)$^{-1}$. In concentrations ranging from 7.6×10^1 to 7.6×10^6 CFU (50 µl)$^{-1}$, there was a linear relationship, with a correlation coefficient of 0.98, between the impedance change and the logarithmic value of S. Typhimurium cells in pure samples. The IDAM imbedded flow cell also had a linear relationship between the impedance change and the logarithmic value of S. Typhimurium cells in contaminated chicken rinse water, with a correlation coefficient of 0.66. The developed portable impedance immunosensor has a LOD comparable with commercial electrochemical impedance instruments. The developed immunosensor also has advantages in portability, low cost, rapid and label-free detection showing a great potential for in-field detection of foodborne pathogens. On going research is focused on the development of a prototype with a IDAM embedded flow cell, an automatic sample and reagent delivery unit, a signal conditional interface, and a laptop computer with LabVIEW software for rapid detection of *S.* Typhimurium in poultry samples.

Acknowledgments

This research was funded in part by Walmart Foundation and was supported by Walmart Food Safety Collaboration Center (002162-00001A), Arkansas Bioscience Institute (0383 43076-24-2333), and the National Natural Science Foundation of China (31401281). The authors thank Lisa Kelso for her help in microbial tests.

References

1. Centers for Disease Control and Prevention. Available online: https://www.cdc.gov/salmonella/typhimurium-live-poultry-04-13 (accessed on 05-18-2017).
2. Lakshmanan, R. S.; Guntupalli, R.; Hu, J.; Petrenko, V. A.; Barbaree, J. M.; Bryan, A. C. Detection of Salmonella Typhimurium in fat free milk using a phase immobilized magenetoelastic sensor. Sens. Actuators B. 2007, 126, 544-550. http:// doi:10.1016/j.snb.2007.04.003