An Electrochemical Aptasensor for Rapid Detection of *Salmonella* Typhimurium in Poultry Based on the Bifunctional Magnetic Nanocomposites

Ronghui Wang[1], Meng Xu[1], Jianhan Lin[3], Ming Liao[4], Michael Kidd[2] and 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]MOA Key Laboratory of Agricultural Information Acquisition Technology, China Agricultural University, Beijing 100083, China

[4]College of Veterinary Medicine, South China Agricultural University, Guangzhou, Guangdong 510642, China

ABSTRACT

In this study, an electrochemical aptasensor based on the magnetic bifunctional polydopamine (PDA) polymeric nanocomposites (PMNCs) was developed for rapid detection of *Salmonella* Typhimurium. The core-shell magnetic beads (MBs)-glucose oxidase (GOx)@PDA PMNCs were first synthesized as the primary vehicle to support the further functionalization and to isolate and concentrate the target bacteria. Gold nanoparticles (AuNPs) were biochemically synthesized on the surface of PMNCs to adsorb anti-*Salmonella* aptamers. The 3, 3'-Dithiodipropionic acid di(N-hydroxysuccinimide ester (DTSP) was used to cross-link the AuNPs and the aptamers. The final product of the aptamers/GOx_{ext}/AuNPs/MBs-GOx@PDA PMNCs can carry the target bacteria and be magnetically attracted to the surface of the screen-printed interdigitated electrode (SP-IDME) for measurement. The redox probe containing glucose could recognize the current changes caused by the attachment of *S.* Typhimurium cells. The synthesized aptamers/GOx_{ext}/AuNPs/MBs-GOx@PDA PMNCs was demonstrated to be able to generate current responses that were linearly related to the log concentration of *S.* Typhimurium. The detection limit of the developed aptasensor was achieved at 96 cfu/ml for *S.* Typhimurium in pure culture within 1 h without any pre-enrichment procedures. Ongoing research will focus on the validation of the aptasensor with poultry samples. The outcome of this study will provide a bifunctional PMNCs to improve the sensitivity of the aptasensor due to the higher loading efficiency of biomaterials through PNMCs.

INTRODUCTION

- *Salmonella* Typhimurium is one of the most commonly identified foodborne pathogens for humans and animals, which pose a threat to human health and cause substantial economic cost to society: (1) people infected with *S.* Typhimurium develop diarrhea, fever, abdominal cramps, and even death; and (2) it is estimated that *S.* Typhimurium is responsible for 1821 illnesses and 197 hospitalizations, resulting in $8 million economic costs each year in the United States.
- Since traditional methods of *S.* Typhimurium detection, such as bacterial culture and polymerase chain reaction (PCR), are time consuming and/or require highly trained personnel and specialized facilities, there is an urgent need for a sensitive and efficient technique to rapid detection of *S.* Typhimurium in foods.

OBJECTIVE

The objective of the present study was to develop an electrochemical aptasensor for rapid detection of *S.* Typhimurium based on the magnetic bifunctional polydopamine (PDA) polymeric nanocomposites (PMNCs).

MATERIALS & METHODS

Apparatus:

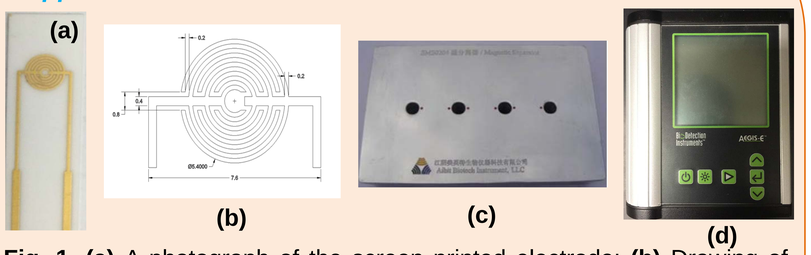

Fig. 1. (a) A photograph of the screen printed electrode; **(b)** Drawing of the interdigitated electrode. Dimensions are given in millimeters; **(c)** Magnetic separator; and **(d)** BDI handheld electrochemical detector.

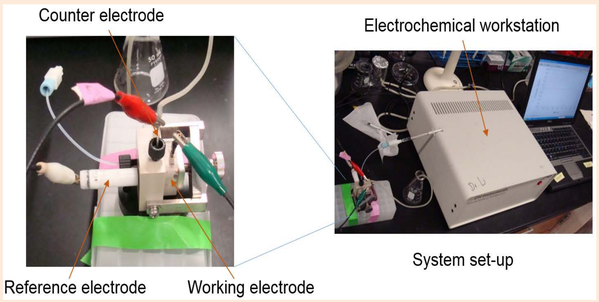

Fig. 2. The three electrodes electrochemical configuration used for measurements. All electrochemical measurements were performed using an electrochemical workstation model 750B.

Materials:

- ***Magnetic Beads (MBs):*** Size: 130 nm; Streptavidin coated, dextran based, 10 mg/ml solid content (Kisker BioTech GmbH & Co. KG).
- ***Antibody:*** Biotin labeled rabbit anti-*S.* Typhimurium antibodies (4-5 mg/ml) (Meridian Life Science).
- ***Bacterial culture:*** *E. coli* O157:H7, *E coli* K12 and *Salmonella* Typhimurium were obtained from American Type Culture Collection with ATCC number 43888, 29425 and 14028, respectively.
- ***Aptamer:*** The aptamer sequence is 5'-CAG TCC AGG ACA GAT TCG CGA GCC CAC TCC AAA CAC GAC CAA CTC ACG CTC TAT CAA CAT CGC TAT CCA CGT GGA TTT CAT TCA GCG ATT-3', and was developed in our group with detailed information described in our previous study.

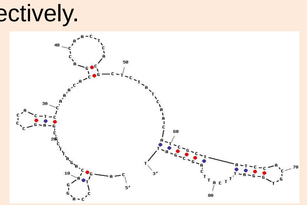

Fig. 3. The secondary structure of the aptamer.

Synthesis of the bifunctional PMNCs:

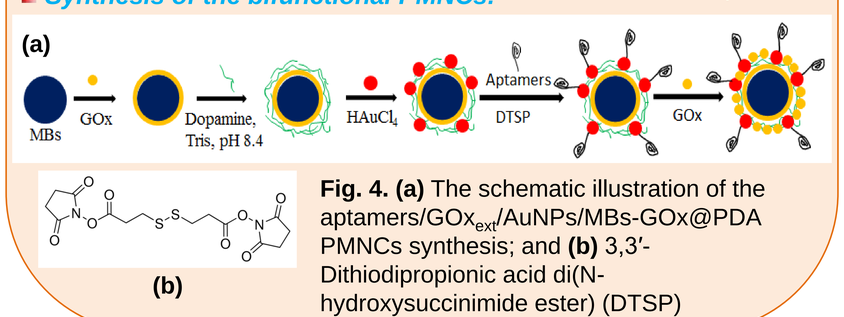

Fig. 4. (a) The schematic illustration of the aptamers/GOx_{ext}/AuNPs/MBs-GOx@PDA PMNCs synthesis; and **(b)** 3,3′-Dithiodipropionic acid di(N-hydroxysuccinimide ester) (DTSP)

RESULTS

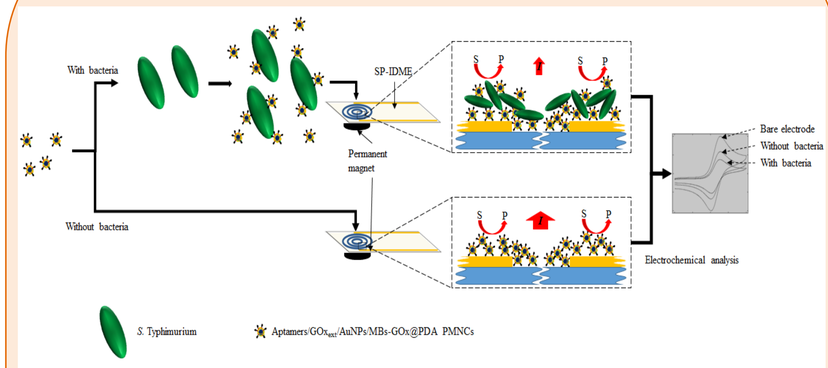

Fig. 5. The construction of the electrochemical biosensor for the detection of *S.* Typhimurium.

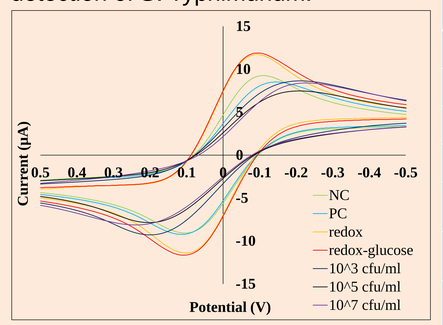

Table 1. The current response at E_{peak} for different analytes.

Sample	E_{peak} (V)	I (μA)
Redox	-0.1	11.68
Redox-glucose		11.88
NC		9.22
10^3 cfu/ml of S. Typhimurium		7.29
10^5 cfu/ml of S. Typhimurium		6.17
10^7 cfu/ml of S. Typhimurium		6.22

Fig. 6. The characteristics of the developed electrochemical aptasensor when testing different analytes.

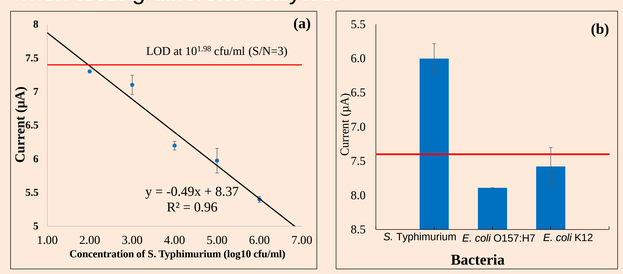

Fig. 7. (a) The calibration curve for detection of *S.* Typhimurium; and **(b)** The specificity of the developed aptasensor for the detection of *S.* Typhimurium.

CONCLUSIONS

- An electrochemical aptasensor based on the magnetic bifunctional PMNCs was developed for rapid detection of *S.* Typhimurium.
- The synthesized PMNCs was able to generate current responses that were linearly related to the log concentration of *S.* Typhimurium.
- The detection limit of the developed aptasensor was achieved at 96 cfu/ml for *S.* Typhimurium in pure culture within 1 h without any pre-enrichment procedures.

ACKNOWLEDGMENTS

This research was supported in part by Arkansas Biosciences Institute and Walmart Foundation through Food Safety Collaboration Center.