An ultrasensitive, rapid and low-cost immuno-detection platform for bacteria and protein biomarkers using field-effect enzymatic detection

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Abstract

A novel immuno-detection system has been constructed by incorporating the newly invented field-effect enzymatic detection (FEED) technique with the immunosensing technique to demonstrate a novel detection platform. The demonstration consists of the detections of three biomarkers and two bacteria. The detected biomarkers include CA125, PSA in serum and AMACR (a novel marker for prostate cancer) in serum and urine. The PSA and AMACR detections were performed on the femto gram/mL level. The two detected bacteria are *E. coli* and Shigella. *E. coli* was detected in milk and meat juice with detection limits on the order of 10 CFU/mL. Because of the intrinsic amplification provided by FEED, the detection was performed without the culture-based amplification, resulting in a significantly shortened assay time of 1 hr. The detection system was realized on screen printed electrodes (SPE). This method, compared with current detection methods, provides three distinctive advantages: (1) ultrasensitive detection of bacteria (10 CFU/mL) and biomarkers (fg/mL) in complex matrices, (2) real-time/rapid detection (1 hour) of bacteria without the culture process, and (3) low-cost detection (<$5/SPE). The patented method can be used as an ultrasensitive, rapid detection platform for point-of-care, disposable and low-cost applications.

1 Introduction

Ultrasensitive, rapid and low-cost detection of bacteria (microorganisms), viruses and protein biomarkers is undeniably a major technological thrust in several areas concerning the daily lives of people today. The mostly noted areas in this regard are food safety and healthcare. The following examples illustrate the global urgency for the need of effective detection technologies. Potentially harmful bacteria were found on 97 percent of chicken breasts bought at stores across the United States (February 2014 issue of *Consumer Reports*). Each year in the United States, 48 million people become sick and 3,000 die from eating tainted food. Contaminated poultry is the leading cause of such deaths, according to the U.S. Centers for Disease Control and Prevention. Infectious diseases are among the most challenging issues facing health care delivery systems around the world today. The World Health Organisation estimates that more than 17 million people die of an infectious disease each year. The United Nations World Health Organization released its 2014 *World Cancer Report*, stating that cancer cases worldwide are expected to increase 57 percent by 2032.

Effective detection methods are urgently needed for the detection of small amounts of analytes with short assay time. The availability of such methods will allow early detection of infectious diseases and cancers so that effective treatment can be conducted. Ideal detection technologies should provide the following basic properties:

(a) Ultra-low detection limit (ultra-high sensitivity) for the detection of species directly in real samples with minimum or without additional sample preparation/manipulation,
(b) A high specificity for the exclusion of the interference in the sample,
(c) Short assay time,
(d) The adaptability to detect different kinds of species,
(e) Low cost, and
(f) Compact size for portable use.

Presently, the realization of an ideal detection technology for on-site or portable early detection of bacterial pathogens remains a challenge for detection technology.

2 Market opportunities

The detection platform described here will serve mainly two markets, namely, the food production/processing industry and the healthcare sector.

Food manufacturing companies perform food safety testing to ensure the safety of their food products. Worldwide regulatory agencies specify that implementation of food safety testing and certification is crucial for the food manufacturing companies. Food safety testing is performed on different types of food products such as meat & poultry products, dairy products, processed foods, fruits & vegetables, and other foods such as cereals, food additives, etc. According to marketing research (MarketsAndMarkets.com), the predicted global food testing sales will exceed $14 billion USD by 2018, up from $9 billion USD in 2012.

Infectious diseases are among the most challenging issues facing healthcare delivery systems around the world today. The World Health Organization estimates

that more than 17 million people die of an infectious disease each year. There has been a global resurgence of infectious diseases, including the identification of new pathogens, the re-emergence of old infectious agents, such as tuberculosis and malaria and cholera, and the rapid spread of antimicrobial resistance. Increasing incidence of infectious disease is expected to make the market for infectious disease among the most dynamic in the in vitro diagnostics industry, according to Kalorama Information. The world market for infectious disease tests was about $14.5 billion in 2012 and is expanding at a fast rate.

Biomarker tests for various diseases continue to impact the treatment of cancer from early detection through diagnosis, by helping patients to better understand their risk of having the disease and identifying the aggressiveness of the disease if present. Frost & Sullivan sized the U.S. cancer biomarker testing market at $7.86 billion in 2011 and expects this to reach $11.46 billion by 2017.

3 The detection platform

Current detection technologies provide unsatisfactory performance and are expensive to use. Conventional methods for the detection of bacteria such as enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) require a series of culture-based amplification steps in order to increase the number of the target in the sample to a detectable level. The amplification process makes conventional methods time- and labor-consuming. Detection of protein biomarkers using ELISA is known to provide unsatisfactory sensitivity.

Recently, the author has invented field-effect enzymatic detection (FEED)[1], a novel bio-sensing technique, in which an external gating voltage V_G is used to provide intrinsic amplification of the signal current of an enzymatic biosensor. Fig. 1a shows the detection setup. A three-electrode electrochemical system is modified by the gating electrodes, which applied the gating voltage V_G to the redox enzyme (green elliptical structures) immobilized on the working electrode. V_G induces ions at the solution-electrode interface to set up an electric field within the enzyme to modulate interfacial charge transfer. The field lowers the tunnel barrier between the electrode and the active center of the enzyme and therefore increases the tunnel current to provide amplification to the signal. The patented quantum mechanics-based technique was used to obtain the detection limit of molecular analytes on the zepto-molar (10^{-21}M) level[2]. Fig. 1b shows that the signal current is amplified by the gating voltage (V_{ext} or V_G) and Fig. 1c shows the detection of glucose on the picomolar level. Without the amplification, detection is possible only on the micromolar level. The novel method has been elucidated in several publications[3, 4].

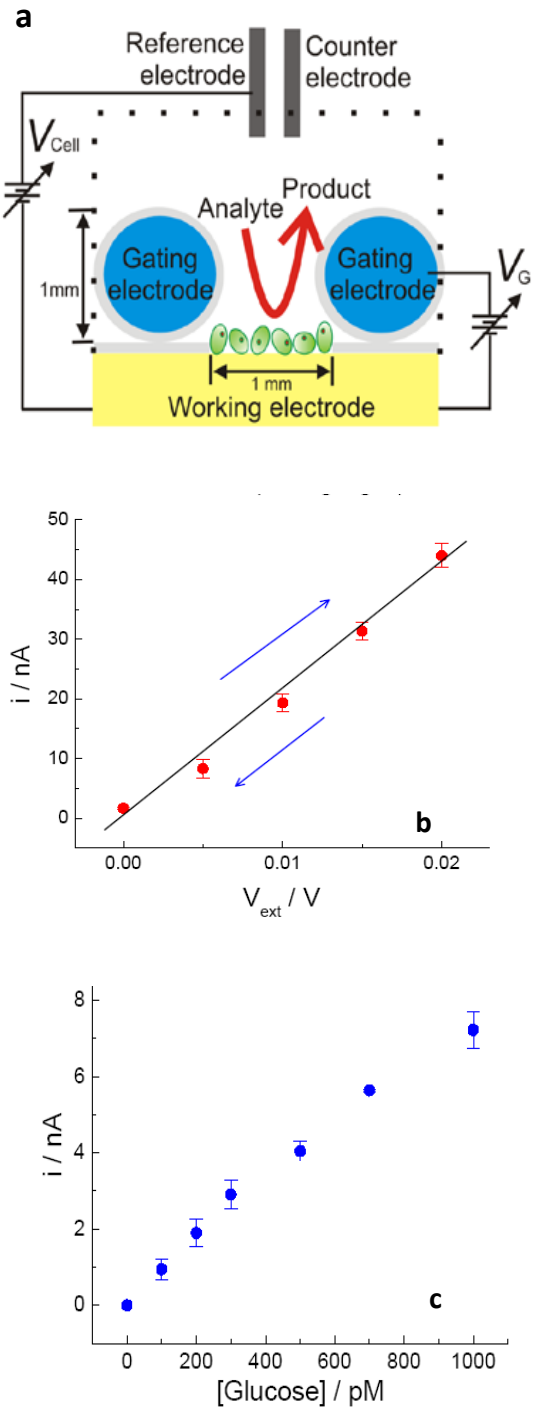

Figure 1

The author has incorporated FEED with the immunosensing technique to demonstrate a novel detection platform. Fig. 2 shows the antibody-antigen-antibody immune complex formed on the working electrode of the FEED system. The enzyme used to label the detecting antibody is immobilized on the electrode

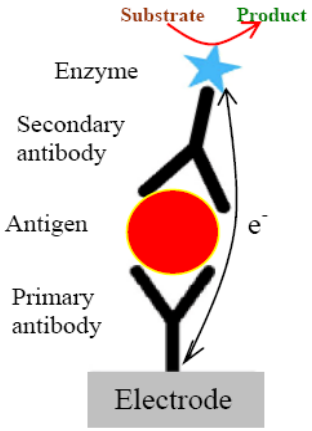

Figure 2

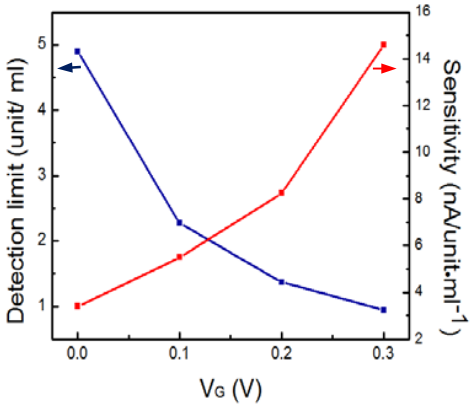

Figure 3

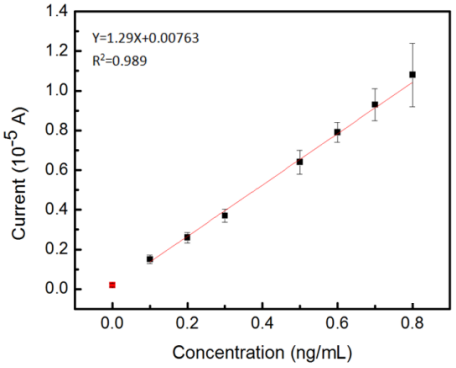

Figure 4

via the complex. Three biomarkers and two bacteria were detected. The detected biomarkers were cancer antigen 125 (CA125, a biomarker of ovarian cancer)[5], prostate specific antigen (PSA, a biomarker of prostate cancer) in serum[6] and alpha-methylacyl-CoA racemase (AMACR, a novel marker of prostate cancer) in serum and urine[7]. The PSA and AMACR detections were performed on the pico-femto gram/mL level. The two detected bacteria were *E. coli* [8] and *Shigella*[9]. *E. coli* was detected in milk and meat juice with detection limits on the order of 10 CFU/mL. Because of the intrinsic amplification provided by FEED, the detection was performed without the culture-based amplification, resulting in a significantly shortened assay time of 1 hr. In these works, FEED provided ultrasensitivity due to its intrinsic amplification whereas the immunosensing technique provided a high degree of substance selectivity. The detection system was realized on screen printed electrodes (SPE), intended for point-of-care, disposable and low-cost applications. Fig. 3 shows the V_G-dependent detection limit and sensitivity observed in the detection of CA 125. As V_G is increased, the detection limit decreases while the sensitivity increases. Fig. 4 shows AMACR detection calibration curve on the pg/mL level in serum with V_G=0.6 V. The detection was performed with commercial AMACR spiked in serum. The detection limit was 100 pg/mL. Similar result was obtained in urine samples. Figs. 5a and 5b respectively show the detection signal current of PSA in serum and the calibration curve. The detection limit is 58 fg/mL.

Figs.6a and 6b show the result of detection of *E. coli* in milk. The gating voltage of FEED facilitated the transduction of electrical signal through the bulky immune complex so that the detection did not rely on the use of mediators. The voltage-controlled intrinsic amplification provided by the detection system allowed the detection in low-concentration samples without target pre-enrichment, leading to ultrasensitive and rapid detection. The detection approach was demonstrated with E. coli O157:H7, a model microorganism, in milk with an estimated detection limit of 20 CFU/mL without performing sample pre-enrichment and centrifugation of sample followed by the resuspension of the pellet in a buffer solution, resulting in a significantly shortened assay time of 67 min. Optimizing the gating voltage resulted in the detection of 12 CFU/mL of the bacterium in milk.

4 Impact of platform

The infectious doses (ID) of infections are usually low. For example, the ID of *E. coli* O157:H7 is about 10 cells and that of *Shigella* is on the order of 10 cells. Current bacteria detection techniques such as enzyme-linked immunosorbent assay (ELISA) is able to detect small quantities of bacteria only if a series of culture steps (enrichment) is performed in order to increase the number of the bacteria to a detectable level. The culture process is time- and labor-consuming, leading to typical assay times from 24 hours to several days. Detection of protein biomarkers using ELISA is known to provide unsatisfactory sensitivity. The microplate reader used to perform ELISA costs about $ 20,000. Although the most advanced detection technology, the real-time polymerase

chain reaction (RTPCR), provides low detection limit (several cells), it still requires assay times of 1-3 hours and the analytical unit cost about $20,000 while the cost per test is about $15. The operation of PCR in general requires the knowledge of genetics, e.g. DNA and RNA. Both ELISA and RTPCR are desktop-based equipment.

The featured detection platform will provide facelift solutions to the issues mentioned above. The amplification provided by the platform will allow detection of extremely small amounts (<10 cells) of bacteria to be performed without requiring the culture process, leading to significantly shortened assay time (1 hour). The electrochemical nature of the detection approach will result in low-cost (~$100/control unit and ~$5/test), easy-to-use, compact, versatile and disposable detection devices for point-of-care (POC) applications. The concept of the detection approach is similar to that of the glucose test strips. The detection platform provides three distinctive advantages: (1) ultrasensitive detection of bacteria and biomarkers in complex matrices, (2) real-time/rapid detection of bacteria without the culture process, and (3) low-cost detection.

5 Conclusion

The experimental results presented here indicate that the FEED-based immuno-detection system is a versatile platform for the ultrasensitive, rapid detection of biological antigens. Its electrochemical nature allows the platform to be used in low-cost, disposable and POC applications

References

[1] Y. Choi, and S.-T. Yau, "A Field-Effect Enzymatic Amplifying Detector with Pico-Molar Detection Limit," *Analytical Chemistry,* vol. 81, pp. 7123-7126, 2009.

[2] Y. Choi, and S.-T. Yau, "Ultrasensitive biosensing on the zepto-molar level," *Biosensors & Bioelectronics,* vol. 26, pp. 3386–3390, 2011.

[3] Y. Choi, and S.-T. Yau, "Field-controlled electron transfer and reaction kinetics of the biological catalytic system of microperoxidase-11 and hydrogen peroxide," *AIP ADVANCES* vol. 1, pp. 042175, 2011.

[4] S.-T. Yau, Y. Xu, Y. Song *et al.*, "Voltage-controlled enzyme-catalyzed glucose–gluconolactone conversion using a field-effect enzymatic detector," *Phys.Chem. Chem. Phys.,* vol. 15, pp. 20134-20139, 2013.

[5] J. Wang, and S.-T. Yau, "Field-effect amperometric immuno-detection of biomarker," *Biosensors and Bioelectronics,* vol. 29 pp. 210– 214, 2011.

[6] J. Wang, and S.-T. Yau, "Detection of Prostate Specific Antigen in Serum at the Femto-gram Per Milliliter Level Using the Intrinsic Amplification of a Field-effect Enzymatic Immuno-sensing System," *Electrochimica Acta,* vol. 111, pp. 92-98, 2013.

[7] J. Wang, and S.-T. Yau, *Electroanalysis*, accepted.

[8] J. Wang, Y. Xu, and S.-T. Yau, "Mediator-less immunodetection with voltage-controlled intrinsic amplification for ultrasensitive and rapid detection of microorganism pathogens," *ChemElectroChem*, accepted, 2013.

[9] J. Wang, and S.-T. Yau, in preparation.

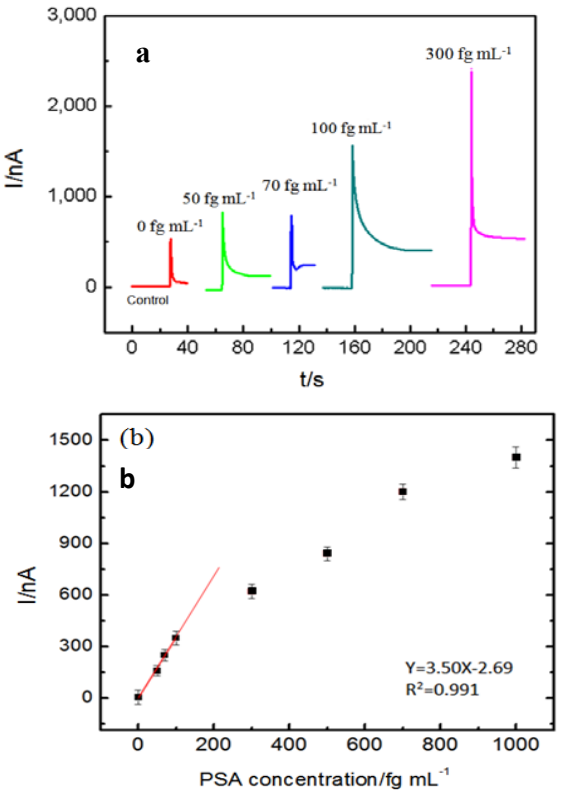

Figure 5

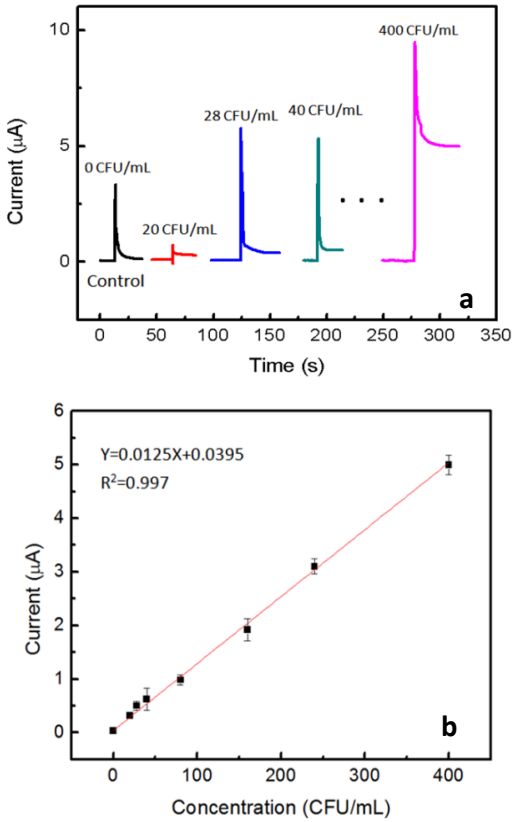

Figure 6