Ultra- sensitive Point Of Care biosensor for detecting pathogeneses

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Problem Statement : Bacterial pathogens in food products have recently caused a number of public health issues from e-coli and salmonella outbreaks . These infections are traditionally detected using time consuming and labor intensive conventional methods [1,2]. Various techniques have been developed for Deoxy Ribonucleic Acid (DNA) detection such as Polymerase Chain Reaction (PCR) or antigen-antibody detection (Enzyme Linked Immuno Assay- ELISA) but they take 1-2 days to be completed. The time and complexity of the test and increases while the reliability decreases as the sensitivity increases. More sensitive tests are a precursor for early pathogen detection.

Objective : In this paper we study the sensitivity in the detection of e-coli using a patented Bionanosensor [3] . The total testing time using this sensor is l0-20 minutes . We also compare and contrast this sensitivity data with tests performed by the traditional method (q-PCR).

Methods: This novel Bionanosensor works by immobilization of a single-stranded (ss) DNA probe that recognizes a specific complementary target ss-DNA in a sample solution and converts it to the double stranded (ds)-DNA (known as hybridization) into a useful analytical signal. On analysis of this signal , performed a few minutes after the addition of the sample, we can test if the sample has been hybridized with the DNA-probe or not (Non-Hybridization). Hybridization means the presence of pathogens or positive test and non-hybridization is absence of pathogen and negative test. In this study, different concentrations of *E.coli* ssDNA from 10 picogram to 1 femtogram were added to form a solution with TE-Buffer (100 nM to 10pM by performing serial dilution) were tested with a immobilized complimentary ss-DNA (5' CCG ATA CGC TGC CAA TCA GT 3') and reverse (5' CAC ATG GCC AAC CCA ACA AA 3') . 4 sensors were measured simultaneously. The first sensor is always used as the negative control. The voltage and resistance across each biosensor were measured with a self-designed, feed-back controlled, balanced Wheatstone bridge circuit with an Arduino interface. This voltage/resistance data was averaged over 3 similar experiments or 9 biosensor chips in-situ using a Python Code. Since the hybridization is very temperature sensitive, the measurement was performed on a thermally insulated temperature controlled device. To validate the measurements, qPCR was used to validate the time and cycles of *E.coli* genome at the concentrations of *E.coli* genome that were tested on the sensors.

Results:

The sample is pipetted onto the sensors 300 seconds after the *Ecoli* ss-DNA primer has been primed to the sensor . If there is hybridization, the voltage/resistance of the sensor drops due to the decrease in conductivity. If there is no hybridization then the resistance remains constant. The consistent difference in voltage pattern, measured across the sensor , measured 200 seconds from the injection of the sample is the basic test for determining hybridization or positive test and non-hybridization or negative test. This difference was observed as the sample DNA was serially diluted from 100 nanoMolar(nM) to 1 picoMolar(pM). This is a direct and very simple

test without the necessity of sample amplification. No sample amplification was required in this test and hence. The hybridization/non-hybridization processed data obtained for sample sets of 9 each is provided below. The sensitivity of the sensors was compared with the traditional qPCR and the sensitivity of qPCR was determined in the concentration of 4.66 pg that also demonstrated an overlap with 466 fg. The number of cycles to amolify the *uspA* gene in concentration of 466 fg by qPCR was achieved in 30 cycles. In comparison with the sensitivity of the sensors, the 1pmol (27.5 fg) of the same ssDNA primers used in qPCR were detected by changing the voltage in hybridization by 20 mV. From the obtained data, the number of *E.coli* in the sensitivity of detection were also calculated based on the "DNA copy number = moles of ssDNA x $6.022e^{23}$ molecules/mol" . DNA copy number of *E.coli* in concentration of 27.5 fg of ssDNA (in comparision of the E.coli genome with 5000,000 bp) was calculated and the 10 number of *E.coli* was achieved in that concentration. While in traditional qPCR the highest sensitvity can detect less than 600,000 number of *E.coli*.

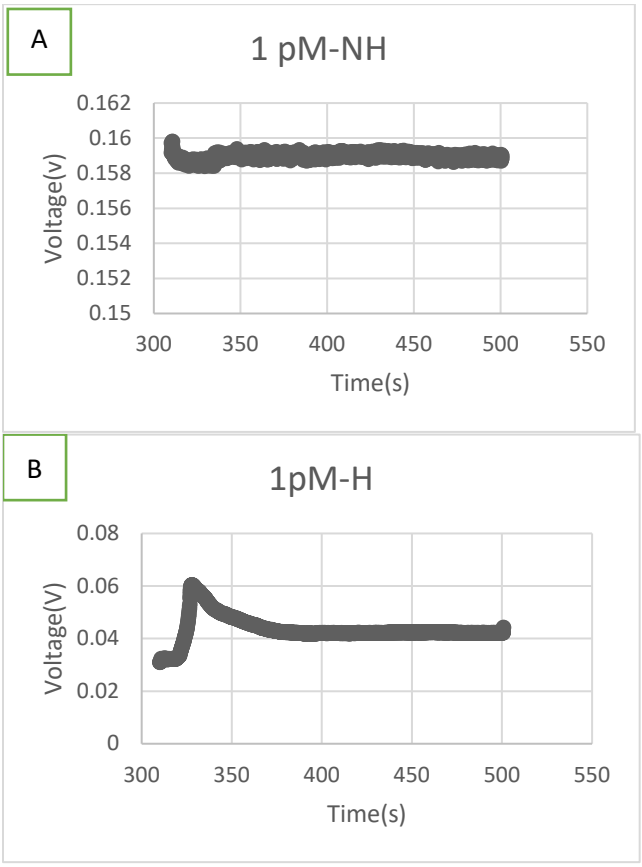

Figure1; Non-hybridization *(A)* and hybridization *(B)* voltage change of 20 bp ssDNA *E.coli uspA* on sensors. The concentration of the DNA is 1pM. Voltage measurements data were normalized and averaged in both experiments of hybridization (n= 9) and non-hybridization(n=9). The voltage change after the reaction of forward and reverse primers from 300 second to 500 second were shown in the above graphs.

Conclusions

In this study we have demonstrated an ultra- sensitive biosensor which can detect the genome of pathogeneses diseases in low concentrations up to femto-gram (which is comparable to the weight of 10 *E-coli* bacteria). In traditional methods such as qPCR we may can detect concentrations of DNA this low, but by performing more than 35 cycles of amplification which results in longer time (1 day) for analysis as well extremely unreliable data . Thus, we have demonstrated a biosensor which can detect in 10 minutes the likelihood of bacterial infection at a very early stage with a sensitivity of detecting few single *E-coli* bacteria.

References:

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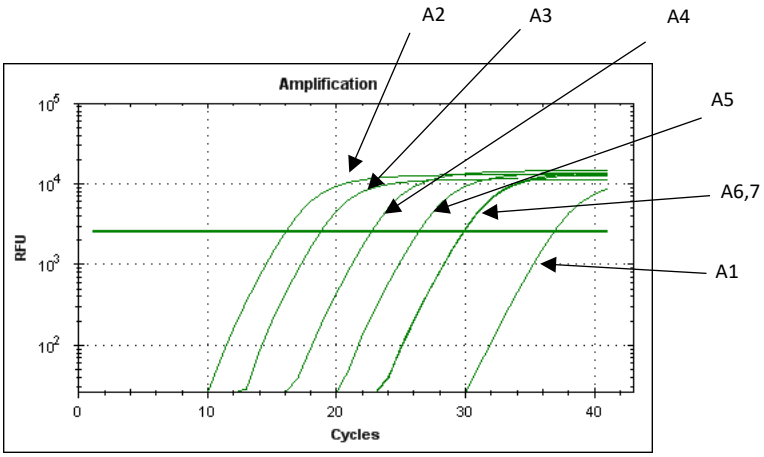
2. Hong Cai · Xuni Cao · Ying Jiang · Pingang He · Yuzhi Fang Carbon nanotube-enhanced electrochemical DNA biosensor for DNA hybridization detection,Anal Bioanal Chem (2003) 375:287–293 DOI 10.1007/s00216-002-1652-9, 4 January 2003.
3. US Patent # 9919922 “Bionanosensor Detection Device”, 2018



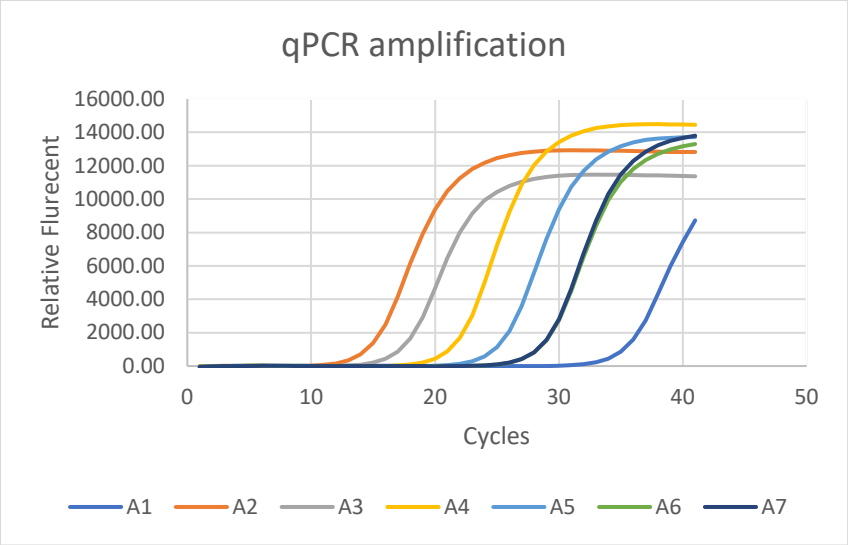


Figure2; Amplification of *E.coli uspA* gene by qPCR. The qPCR of *E.coli uspA* gene was performed in six sample with different concentrations included; A2= 46.6 ng, A3=4.66 ng, A4=466 pg, A5=46.6 pg, A6=4.66 pg, A7= 466 fg, A1=0 (negative control with no E.coli template genome).