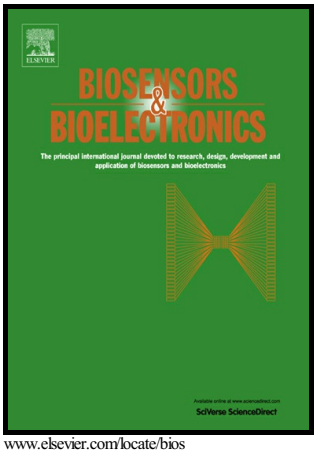

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Sensitive detection of multiple pathogens using a single DNA probe

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Abstract

A simple but promising electrochemical DNA nanosensor was designed, constructed and applied to differentiate a few food-borne pathogens. The DNA probe was initially designed to have a complementary region in *Vibrio parahaemolyticus* (VP) genome and to make different hybridisation paterns with other selected pathogens. The sensor was based on a screen printed carbon electrode (SPCE) modified with polylactide-stabilized gold nanoparticles (PLA-AuNPs) and methylene blue (MB) was employed as the redox indicator binding better to single-stranded DNA. The immobilization and hybridization events were assessed using differential pulse voltammetry (DPV). The fabricated biosensor was able to specifically distinguish complementary, non-complementary and mismatched oligonucleotides. DNA was measured in the range of 2.0×10^{-9} - 2.0×10^{-13} M with a detection limit of 5.3×10^{-12} M. The relative standard deviation for 6 replications of DPV measurement of 0.2 µM complementary DNA was 4.88 %. The fabricated DNA biosensor was considered stable and portable as indicated by a recovery of more than 80 % after a storage period of 6 months at 4 - 45 °C. Cross-reactivity studies against various food-borne pathogens showed a reliably sensitive detection of VP.

Keywords

Electrochemical DNA biosensor; polylactide-stabilized gold nanoparticles (PLA-AuNPs); Nucleic acid hybridization detection; methylene blue; food-borne pathogens

1. Introduction

New rapid and sensitive methods for the detection of pathogenic bacteria continue to be developed and to offer potential application in such areas as biomedical diagnosis, food safety and

environmental monitoring (Fernandes et al. 2015). These can be broadly grouped into either nucleic acid-based or antigen-based methods (Law et al. 2014). It is the first group which is of relevance to this study. DNA detection, in particular, is attracting a great deal of attention across a number of fields with chemical, physical and biological investigations being tried (Ahn et al. 2015; Cai et al. 2014; Chernev et al. 2014; Waldner et al. 2013; Xue-tao et al. 2015; Yu et al. 2015). The most popular DNA biosensors are optical or electrochemical (Velusamy et al. 2010; Xihong et al. 2014). Many researchers have reported the successful detection of foodborne pathogens by electrochemical biosensors (Pal et al. 2008) and they show promise simple, rapid detection, specific and selective with a low cost for mass fabrication (Ding et al. 2013; Lazerges and Bedioui 2013; Manzanares-Palenzuela et al. 2015; Rahman et al. 2015; Zhao et al. 2013).

A dynamic influence in the field of electrochemical biosensors has been the introduction of nanotechnology. The unique features of nanomaterials (their size, surface characteristics and inner structure) have opened up extensive ways for electrode surface modification (Karimi-Maleh et al. 2016; Karimi-Maleh et al. 2013; Karimi-Maleh et al. 2014; Zhao and Boström 2016). Gold nanoparticles (AuNPs), for example, have been shown to improve the construction of DNA biosensors by increasing the active surface area and conductivity (Huang et al. 2014b; Mohammed et al. 2014; Sedighi et al. 2014; Shi et al. 2013; Xu et al. 2013). Among the polymers used for surface modification, polylactide (PLA) appears to contribute superior properties such as biodegradability. To date, there are few reports on the application of PLA-AuNPs in DNA biosensor fabrication although there certainly have been some successful attempts. Wu et al. (Wu et al. 2011) demonstrated that their Au/PLA nanocomposite modified electrode was able to offer a superior hydrophilic interface for the rapid and highly selective identification of leukaemia cancer cells. Song et al. (Song et al. 2006) demonstrated that the detection of As(III) using SPE/PLA–AuNP can selectively differentiate intervention of Cu, Cd, Fe, Zn, Mn and Ni in natural waters and therefore provides a selective, direct and sensitive detection technology to be practically used. Han et al. (Han et al. 2014) demonstrated that the detection of hydrogen peroxide using MWCNT-g-PLA-Pd can selectively differentiate intervention of uric acid, ascorbic acid and glucose in blood samples and therefore provides a stable and selective detection technology to be practically used.

These few examples alone are sufficient to motivate further exploration and thus, in this study, we conducted a detailed investigation into the performance of a developed DNA biosensor by using a more stable electrode modified with AuNPs.

As our target, we selected *Vibrio parahaemolyticus* (VP), a food-borne pathogen which can cause acute gastroenteritis characterized by nausea, headache, diarrhea, vomiting and abdominal cramps (Costa Sobrinho et al. 2014; Letchumanan et al. 2014). This gram-negative bacteria is

recognized as the leading cause of human gastroenteritis associated globally with seafood consumption (Zhang and Orth 2013). The three main virulence factors associated with VP are thermostable direct hemolysin (TDH), thermostable direct hemolysin-related hemolysin (TRH) and thermolabile hemolysin (TLH) (Gutierrez West et al. 2013). The appearance of the TDH gene carried by the O3:K6 serotype is adversely impacting on export trade and is accountable for most outbreaks worldwide (Ceccarelli et al. 2013). Generally, three primers which specifically target the VP-specific toxR gene (Vp-toxR), TDH and TRH hemolysin genes are widely used for VP identification (Han et al. 2015; Hossain et al. 2013). Research into early detection of VP has attracted an abundance of interest, particularly in China where monitoring for the pathogen is included in the country's seafood plan (Wang et al. 2015). Currently, the most commonly used detection method is Most Probable Number (MPN) which has been recommended by the US Food and Drug Administration since 2004. As this is a time-consuming and labour-intensive method (Di Pinto et al. 2008), other techniques have also been tried more recently. These include VP detection through enrichment and selective media including latex agglutination testing, enzyme linked immunomagnetic sorbent assays (ELISA), pulsed field gel electrophoresis (PFGE), polymerase chain reaction (PCR) (Rosec et al. 2009; Wang and Levin 2006) and biosensor technology. Some notable examples are the study of Kumar et al (Kumar et al. 2011) who detected VP in seafood with sandwich ELISA and a subsequent 2014 study by Wang et al (Wang et al. 2015) which used a double-layer agar plate (DLAP) comparing favourably with prior MPN and direct-plating methods in sensitivity and exactness.

The process we used for the detection of VP is clearly depicted in the schematic diagram below (Fig. 1.) Gold nanoparticles (AuNP) are employed to increase the active surface area of the working electrode (WE) while the surface modification is mediated by PLA. MB used as the redox complex turns to leucomethylene blue (LB) after oxidization. Of particular interest, it should be noted that a stronger affinity between MB and single stranded DNA is evident (Farjami et al. 2010). Hybridisation of ssDNA with its complementary sequence replaces the bond MB molecules and thus reduces the current in a differential pulse voltammetry.

Fig. 1. Schematic drawing of electrochemical DNA biosensor based on polylactide-stabilized gold nanoparticles modified electrode.

We further investigated the performance of the developed biosensor with methylene blue (MB) as a redox label (Lin et al. 2015; Zhang et al. 2014). In most documented electrochemical detection

methods, the redox complex that is used binds to double stranded DNA better than single-stranded (Dutse et al. 2014; García-González et al. 2014; Hushiarian et al. 2015a) but in this experiment we used MB that binds to single-stranded better than to double-stranded (Kara et al. 2002; Kerman et al. 2002; Pan et al. 2007). MB acts as a photosensitizer being able to interact easily with DNA due to its planar structure. MB can noncovalently attached to DNA in three ways: electrostatic binding, groove binding and intercalative binding (Vardevanyan et al. 2013a; Vardevanyan et al. 2013b). According to previous research, intercalation is the dominant binding mode, particularly where there are more guanine bases in the DNA, the interaction between the guanine bases occurring mainly in ssDNA (Canete-Rosales et al. 2014; Zhou et al. 2013). MB undergoes rotational transitions inside the intercalative pocket which gives rise to three MB/DNA adducts with different energetic and structural features at different ionic strengths (Nogueira and González 2014).

Another interesting benefit of using MB is that the electrochemical reaction happens at comparatively low potential where interference from other electroactive species and background can be minimized in comparison with what usually occurs in label-free electrochemical methods (Jiang et al. 2015).

2. Materials and methods

2.1. Reagents and apparatus

We investigated hybridization between probe and synthetic oligonucleotides by DPV using a µAutolab III (Eco-chemie, Netherland) voltammetric analyser with General Purpose Electrochemical System (GPES 4.9) software. All the chemicals used here were of analytical grade and purchased from Sigma-Aldrich.

2.2. Preparation of AuNPs, PLA-AuNPs and modified electrode

We synthesised AuNPs following the method reported by Sasha and Hadi (Nasir and Nur 2008). Briefly, this involved heating 100 ml of aqueous chloroauric solution (1 mM) to boiling and quickly adding 10 mL of sodium citrate solution (38.8 mM). The colour of the solution first changed from yellow to blackish and finally to dark ruby red. It was then cooled to ambient temperature. To prepare the PLA sheet, we placed PLA pellets in a preheated stainless steel mould for 10 minutes to melt at 190 °C and then pressed them under a pressure of 2.2 Mpa for 1 minute. The melted PLA was cooled. After cooling, we cut about 0.68 mg of the PLA sheets into small pieces, and dissolved them in 5 ml of chloroform, $CHCl_3$, stirred vigorously at room temperature until completely

dissolved. Next, we mixed in 10 mL of $HAuCl_4$ solution (0.83 mM) and stirred the solution at room temperature until it was completely homogenous. Finally, a 25 µl of homogenous solution of PLA-AuNPs was quickly pipetted on to the SPCE and air dried it for 24 hours before using it.

2.3. **Probe preparation**

We selected the sequences of ssDNA probe and complementary DNA by exploring the National Center for Biotechnology Information (NCBI) database. Synthetic oligonucleotides (20-mer ssDNA probe, 20-mer complementary DNA, 20-mer mismatched DNA and 21-mer non-complementary DNA) were purchased (as lyophilized powder) from First BASE Laboratories, Malaysia with the following sequences:

thiolated ssDNA probe:	5′- / 5ThioMC6-D/CGG ATT ATG CAG AAG CAC TG - 3′
complementary DNA:	5′ - CAG TGC TTC TGC ATA ATC CG - 3′
one-base mismatched DNA:	5′ - CAG TGC TTC TGC **İ**TA ATC CG - 3′
three-base mismatched DNA:	5′ - CAG TGC TTC T**ĊT İ**TA ATC CG - 3′
non-complementary DNA:	5′ - CGC ACA AGG CTC GAC GGC TGA - 3′

We prepared stock solutions of all oligonucleotides (100 µmol l^{-1}) with sterile TE solution (10 mM Tris–HCl, 1 mM EDTA, pH 7.5) divided into analytical portions and kept at -4 °C. The appropriate dilutions were made as they were needed for use.

2.4. **Immobilization and hybridization experiments**

We used SPCE modified with PLA-AuNPs, denoted as SPCE\PLA-AuNPs for this study. A drop casting method was used for DNA immobilization and hybridization. We immobilized a 25 µl of thiolated ssDNA probe (1.2 µM) on the SPCE\PLA-AuNPs and air dried it for 24 hours at room temperature. Then we pipetted 25 µl of different concentrations of complementary DNA on to the SPCE\PLA-AuNPs\ssDNA for 40 minutes at room temperature. We studied the surface morphology of the SPCE prior to modification using scanning electron microscopy (SEM, JEOL JSM 6400). Further studies of hybridization time, temperature and pH were conducted using the optimum concentration of complementary DNA.

2.5. **Electrochemical measurement**

For this work we used an SPCE comprising a three electrode system: a carbon working electrode, a counter electrode and an Ag/AgCl reference electrode. We immersed the electrode in

20 µM MB for 30 minutes, washed it with 0.5 M PBS / 20 mM NaCl (pH 4.5) and rinsed it with deionized water prior to measurement. The same procedure was applied for all interactions including probe DNA, complementary DNA, mismatched DNA and non-complementary DNA samples. We performed electrochemical impedance spectroscopy (EIS) using a potentiostat (Autolab M101) with a frequency ranging from 1 Hz to 2000 kHz at AC amplitude of 5 mV. We conducted DPV using µAutolab III (Eco-chemie, Netherland) and General Purpose Electrochemical System (GPES 4.9) software. DPV measurements of the MB electrochemical reduction were taken in the potential range from -0.5 V to 0.25 V at the step potential of 0.005 V and the modulation amplitude of 0.05 V with a scan rate of 7.73 mVs^{-1} in 0.1 M PBS (pH 7) containing no indicator. We subsequently studied the sensitivity, reproducibility and heat stability of the fabricated DNA biosensor. All reported results were the measurement of the mean value from three replicates.

2.6. Preparation of DNA samples for cross-reactivity study

The following strains were acquired from the Microbial Food Safety and Quality Laboratory, Universiti Putra Malaysia (UPM): *Vibrio parahaemolyticus* (VP), *Klebsiella pneumoniae* (KP), *Listeria monocytogenes* (LM), *Salmonella typhimurium* (ST) and *Campylobacter jejuni* (CJ). We inoculated isolates into a growth broth with 20 % glycerol and stored at −60 °C. We then prepared fresh working culture as we needed it. We isolated genomic DNA from bacteria by a modified boiled lysis method (Ivanov and Bachvarov 1987) and determined its purity and quantity using a biophotometer. The DNA was denatured at 92 °C for 2 minutes and rapidly cooled it in iced water prior to application in the biosensor.

3. Results and discussion

3.1. Surface morphology of modified SPCE

We investigated the size of the nanoparticles and their successful coating with PLA using TEM, Fig. 2a and 2b respectively. Then we used SEM to study the surface topography of the SPCE before (Fig. 2c) and after surface modification with AuNPs (Fig. 2d) and PLA-AuNPs (Fig. 2e). It should be noted particularly that Fig. 2c shows the SEM image of the bare SPCE, Fig 2d shows that in some areas AuNPs have agglomerated and Figure 2e shows the electrode entirely covered.

Fig. 2. TEM images of a) AuNPs and b) AuNPs coated with PLA. SEM images of c) bare SPCE, d) SPCE/AuNPs and e) SPCE/PLA-AuNPs

This initial partial agglomeration shown in our figures has been comprehensively reported in earlier experiments which have confirmed the tendency of gold nanoparticles (AuNPs) to aggregate and lack stability when exposed to certain conditions such as those usually associated with DNA detection assays. To overcome this, PLA can be used to stabilize AuNPs. Evidence of the stability between polymers and AuNPs is contained in the work of Wu et al (2011) who found that as the amount of nanocomposite increased, the current increased to an optimum level where increasing the volume of the nanocomposite decreased the conductivity. They concluded that on the surface of the electrode, PLA forms a nanofiber network that stabilizes the AuNPs and therefore increases the signal. However, a larger amount of the polymer blocks the AuNPs and results in lower current.

3.2. **The electrochemical characteristics of modified SPCE**

We discovered that the impedance of the electrode was abruptly decreased in the presence of PLA-AuNPs nanocomposite (Fig. 3a). This is perhaps due to enhancement of electron exchange between $K_3[Fe(CN)_6]^{3-/4-}$ in the solution and on the electrode surface. The trend of charge transfer resistance (R_{ct}) of DNA during immobilization and hybridization on the modified SPCE depicted in Fig. 3a was consistent with the DPV measurement displayed in Fig. 4a. It can be seen that the R_{ct} of the hybridized DNA (Fig. 3a blue line) is higher than that of the immobilized probe (Fig. 3a red line). Figure 4a confirms this by showing a lower current when DNA is hybridized.

We found that SPCE modified with PLA-AuNPs gave the best peak enhancement from CV results obtained in 0.1 M PBS (pH 7) blank solution after immersion in 20 μM MB for 30 minutes. In parallel, the same measurement trend can be seen using the DPV technique as depicted in Fig. 3b. The highest DPV peak current was obtained by modifying SPCE with PLA-AuNPs at 2.56 μA, this being almost 3 times higher than SPCE\AuNPs at 0.95 μA. Specifically, when bare SPCE was modified with PLA-AuNPs, the peak current of MB increased very obviously from 0.62 μA to 2.56 μA. However, in the presence of AuNPs, the DPV peak current of MB increased slightly in comparison with bare SPCE.

As previously mentioned, of particular interest is the observation that PLA-stabilized AuNPs have better conductivity in comparison with AuNPs when applied to an electrode as is shown in Fig. 3b. As further explanation, the EIS, the semicircle portion observed at high frequencies corresponds to the electron transfer limiting process. The electron transfer resistance (Ret) can be directly measured as the semicircle diameter. This result tallies with the Ret value of the bare SPCE which is 1932 Ω and where the modification of SPCE/AuNPs decreases it to 1730 Ω, further decreasing to 1444 Ω when modified with PLA-stabilized AuNPs.

Fig. 3. Electrochemical study of the surface modification and optimization of the hybridization conditions. a) Impedance spectra of modified electrodes in 1.0 mmol L^{-1} $K_3[Fe(CN)_6]$. b) DPV curves of different SPCE modification after incubation in MB in 0.1 M PBS (pH 7) and c) Optimum time, d) Optimum temperature and e) Optimum pH for DNA hybridization.

3.3. **Optimization of experimental conditions**

We also investigated the relationship between different DNA concentrations with hybridization time, temperature and pH. These conditions need to be optimized as they influence the peak current of DPV. The concentrations of complementary DNA used were as follows:

$$0.2\ \mu M > 0.4\ \mu M > 0.6\ \mu M > 0.8\ \mu M > 1.2\ \mu M > 1.0\ \mu M$$

Overall, the DPV peak current was higher where lower concentrations of complementary DNA were used. As is shown in Fig. 3c, the maximum DPV peak current was obtained at about 10 minutes hybridization time and thus we chose this duration as the optimized hybridization time throughout our experiment. In relation to hybridization temperature, 35 °C gave the highest DPV peak current and thus was selected this as the optimized hybridization temperature for subsequent experiments (Fig. 3d). The optimum pH of the solution (PBS) for the hybridization reaction was 7 (Fig. 3e).

3.4. **The selectivity of electrochemical DNA biosensor**

Fig. 4a shows the DPV results using MB as the detection label for immobilized probe DNA, hybridization of complementary DNA, one-base mismatched DNA, three-base mismatched DNA and non-complementary DNA oligonucleotides. MB has a strong bond with ssDNA of immobilized probe DNA on the modified electrode surface as is represented by a large DPV peak current. After hybridization with non-complementary DNA, the DPV signal decreased to almost half of the immobilized probe DNA current. This may have been due to a smaller amount of MB gathered on the SPCE\PLA-AuNPs\dsDNA surface caused by an unreachable interaction between guanine bases and MB. Interaction modes of MB and DNA are known to be highly affected by such experimental conditions as temperature, pH, DNA concentration, type of buffer and ionic strength (Heng et al. 2013; Ning et al. 2014). Groove and intercalative are usually the most common binding modes of MB to dsDNA leading to accumulation of MB on the surface of dsDNA (Xu et al. 2015). We consistently observed more depleted DPV peak currents when the capture probe DNA was exposed to three-base mismatched DNA and one-base mismatched DNA, respectively. This trend continued for complementary target DNA. The percentage of selectivity rate for MB peak current obtained is

shown in supplementary Table S1. The complementary DNA exhibited the lowest peak current (0.73 µA) among the probe DNA (4.79 µA), non-complementary DNA (3.25 µA), 3-base mismatched DNA (1.05 µA) and 1-base mismatched DNA (0.94 µA). It was obvious that peak current from the target DNA was much smaller than that of other oligonucleotide sequences. This result indicates that the biosensor could be used to discriminate the complementary DNA from the 1-and 3-base mismatched and the non-complementary DNA with high sensitivity and specificity. In summary, the constructed DNA biosensor had a high selectivity of hybridization detection by immobilized probe DNA on the SPCE\PLA-AuNPs' surface.

Fig. 4. a) Histogram of the MB reduction peak current using different types of DNA in 0.1 M PBS (pH 7). Inset is DPV voltammograms of the same conditions. b) Histogram of DPV peak current of different DNA concentrations and c) The plot of logarithmic reduction peak current of MB against logarithmic value of different DNA concentrations. d) Heat stability study after 6 months of storage interval at different temperature.

3.5. Sensitivity of the SPCE\PLA-AuNP\ssDNA

We conducted further investigation of the sensitivity of the constructed DNA biosensor using a DPV method with MB as the indicator and varying the concentration of the complementary target oligonucleotide. In Fig. 4b, it can be seen that the DPV peak current of MB on the SPCE\PLA-AuNPs kept on decreasing as the concentration of complementary DNA increased. A linear correlation between the logarithmic value of MB reduction peak current and the logarithmic value of various target DNA concentrations (0.2 pM to 0.02 µM) is depicted in Fig. 4c, with a linear regression coefficient of 0.959. In our experiment the peak current for the blank (DNA probe) was 4.79×10^{-6} A with the standard deviation of 1.253×10^{-7}A. We used this value to calculate how much difference was made in the peak current after the hybridization with a range of concentrations of the complementary DNA. Plotting the graph using the ΔP and employing the 3σ/m formula (Guo et al. 2013; Huang et al. 2014a; Hushiarian et al. 2015b; Kong et al. 2014) (σ, standard deviation of blank solution; m, slope of the linear curve), we calculated the limit of detection for the fabricated DNA biosensor as 5.28 pM. which is higher than the lowest actually measured sample. The result was consistent with the fact that ΔP for 0.2 pM and 2 pM (2.65×10^{-6} A and 2.81×10^{-6} A) with the standard deviation of 1.19×10^{-7} A and 1.06×10^{-7} A respectively could overlap. For the computation of the limit of quantitation for the fabricated DNA biosensor, we used the 10σ/m formula (σ, standard deviation of blank solution; m, slope of the linear curve) and gained a result of 17.6 pM.

The performance of the fabricated DNA biosensor was comparable to the peak current of other findings which have used nanostructured materials to modify electrodes for DNA detection as

shown in Table 1. The constructed DNA biosensor with SPCE\PLA-AuNPs offered a large surface area which enhanced assembly points for probe DNA immobilization and amplified the peak current. (Das et al. 2014; Han et al. 2013; Huang et al. 2015; Li et al. 2015; Niu et al. 2015; Niu et al. 2013; Yi et al. 2014)

Table 1. Performance comparison of the fabricated biosensor with other DNA biosensor

Composition of the electrodes	Detection method	Linear range (M)	Detection limit (M)	References
CoS/AuNPs	DPV	1.0×10^{-9} - 1.0×10^{-12}	7.0×10^{-13}	Huang et al. 2015
AuNPs-MPTS	DPV	5.0×10^{-9} - 1.0×10^{-11}	5.0×10^{-11}	Das et al. 2014
AuNPs/GR	DPV	1.3×10^{-9} - 2.5×10^{-11}	8.3×10^{-12}	Niu et al. 2013
(+)AuNPs	DPV	1.0×10^{-9} - 1.5×10^{-12}	2.6×10^{-12}	Li et al. 2015
AuNPs-ADH-GSs	DPV	5.0×10^{-8} - 3.0×10^{-13}	3.0×10^{-13}	Yi et al. 2014
AuNPs–GO	DPV	1.0×10^{-9} - 1.0×10^{-14}	3.5×10^{-15}	Han et al. 2013
3D AuNPs	DPV	1.5×10^{-6} - 7.0×10^{-9}	2.0×10^{-10}	Niu et al. 2015
PLA-AuNPs	DPV	2.0×10^{-8} - 2.0×10^{-13}	5.3×10^{-12}	This work

3.6. **Heat stability and regeneration of the sensor**

In order to investigate the heat stability of the constructed DNA biosensor, we kept immobilized probe DNA on SPCE\PLA-AuNPs' surface in a sealed aluminum pouch containing silica gels and stored this at temperatures of 45 °C, 25 °C and 4 °C. We used the amperometric responses from complementary DNA to estimate the percentage recovery of signal production for an interval time of 6 months. Fig. 4d shows that the percentage recovery for the fabricated DNA biosensor slowly decreased to not less than 80 % after a storage period of 6 months, indicating that probe SPCE\PLA-AuNPs\ssDNA is highly stable and portable. We assessed the regeneration of the DNA biosensor by repeatedly denaturing complementary DNA. This was done through rapid cooling in an ice bath after incubating the complementary DNA-modified electrode in hot water at 86 °C for 8

minutes. We found that the hybridization activity was maintained at 91 % of its initial response after regeneration 8 times (n = 5, RSD = 4.05 %).

3.7. Cross-reactivity study of the DNA biosensor against various foodborne pathogens

We also applied the constructed biosensor to detect target toxR gene in genomic DNA samples extracted from other foodborne pathogens. Differentiation of five bacteria species was of our interest and the designed DNA probe was based on the information collected from the genebank with accession numbers JX262976.1, CP012021.1, FO834906.1, FN424405.1 and CP000768.1 for VP, LM, KP, ST and CJ, respectively. The 20-mer DNA probe was designed to have 50 % CG ratio with a few CG bases at 5' end. Supplementary table S2 presents the features of the designed probe and hybridization characteristics with the relevant species. When we assessed cross-reactivity of the DNA biosensor towards various foodborne pathogens, the DPV peak current obtained after interaction gave a very clear VP detection as shown in Fig. 5. From supplementary table 2S and figure 5 it can be seen that by decreasing the number of base pairs, the observed DPV signal increases. This is related to more MB molecules being bound to the probes. The location of base pairs is the second important factor. The hybridization of DNA probe with ST and CJ resulted in 13 base pairs in both cases, but we could still detect a difference in current (0.23 μA). 13 base pairs with ST started from nucleotide 3 but in CJ from nucleotide 5. We therefore concluded that higher concentration of MB closer to the 5' end of the probe (electrode surface) contributed to a higher signal where both pathogens had the same number of base pairs with the probe.

Fig. 5. Cross-reactivity study of DNA biosensor against various foodborne pathogens

The fabricated DNA biosensor demonstrated successful and valid discrimination between VP and the other selected foodborne pathogens. The presence of various foodborne pathogens mimicked the environment of the food sample.

4. Conclusions

The application of SPCE\PLA-AuNPs produced a more sensitive electrochemical DNA biosensor using MB as an electroactive indicator. Unlike conventional electrochemical methods in which the detection is based on direct relation between hybridization and concentration of the redox complex (Dutse et al. 2013), here we used a different strategy where concentration of the redox

complex decreases in the presence of complementary sequences, enabling us to differentiate mismatches. Although the sequence-specific nature of the interaction between MB redox complex and DNA has been studied before (Farjami et al. 2010; Liu et al. 2012; Silvestrini et al. 2015), in this work, by coupling an effective surface modification strategy with a smart bioreceptor design we managed to differentiate samples using a single probe. The probe design is the key element to a successful differentiation among the samples. Based on our findings a higher number of mismatches closer to the immobilized end of the probe resulted in a stronger differentiating signal. It seems that higher selectivity is achievable when the redox complex binds more strongly with the unhybridized biorecognition molecule. This may be an innovation worth following up, as well as the development of sequence specific redox complexes.

In principle, this sensitive and highly selective DNA biosensor ought to be able to be taken out of the laboratory to be applied in practice. In addition, its good reproducibility and stability make it potentially suitable for miniaturization and mass production. Overall, the fabricated DNA biosensor successfully demonstrated effective and reliable specific VP detection from various food-borne pathogens.

Conflict of interest

The authors declare that there is no conflict of interest.

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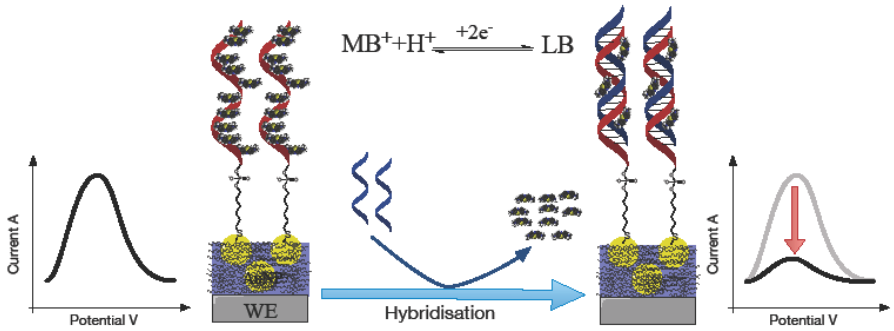
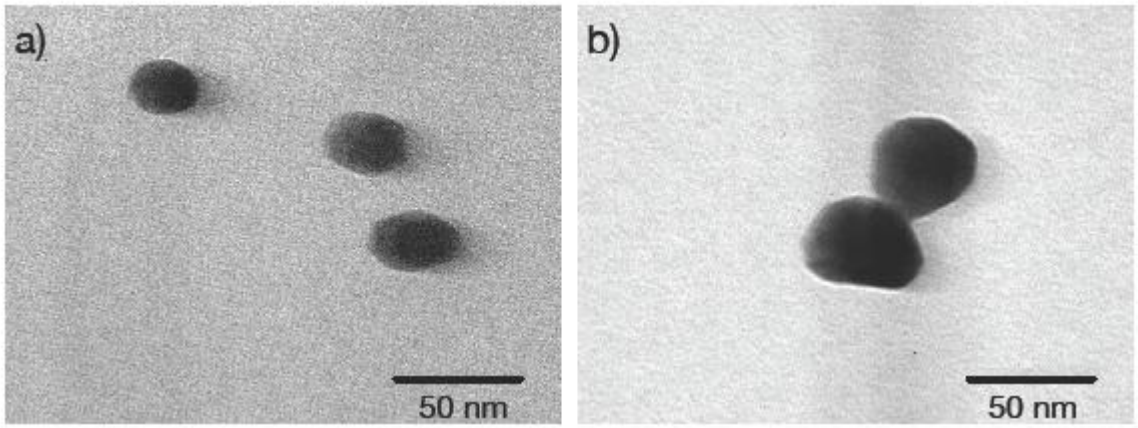
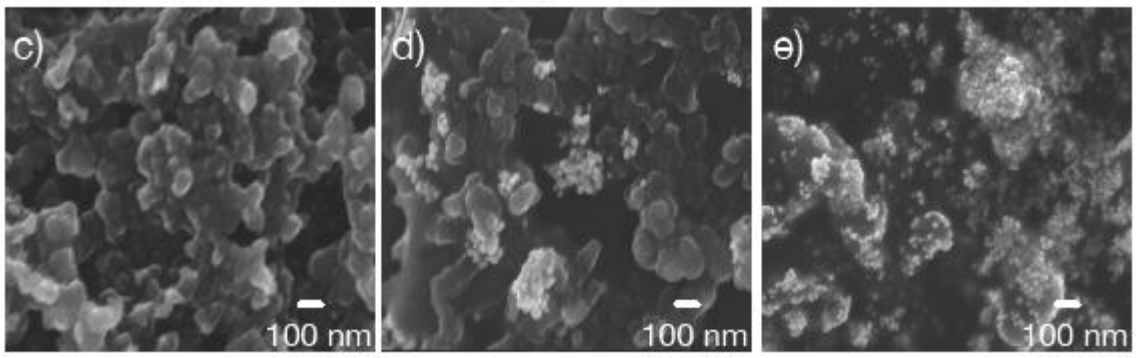
Highlights

- An SPCE modified with PLA-AuNPs was constructed to immobilize a DNA probe.
- The sensor was studied using DPV technique and methylene blue as the redox complex.
- Sensitivity and selectivity of the designed DNA sensor was assessed.
- A few food-borne pathogens were successfully assessed and differentiated.


MB+ +H+ +2e- LB
Current A
Potential V
WE
Hybridisation
Current A
Potential V


a)
50 nm
b)
50 nm


c)
100 nm
d)
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e)
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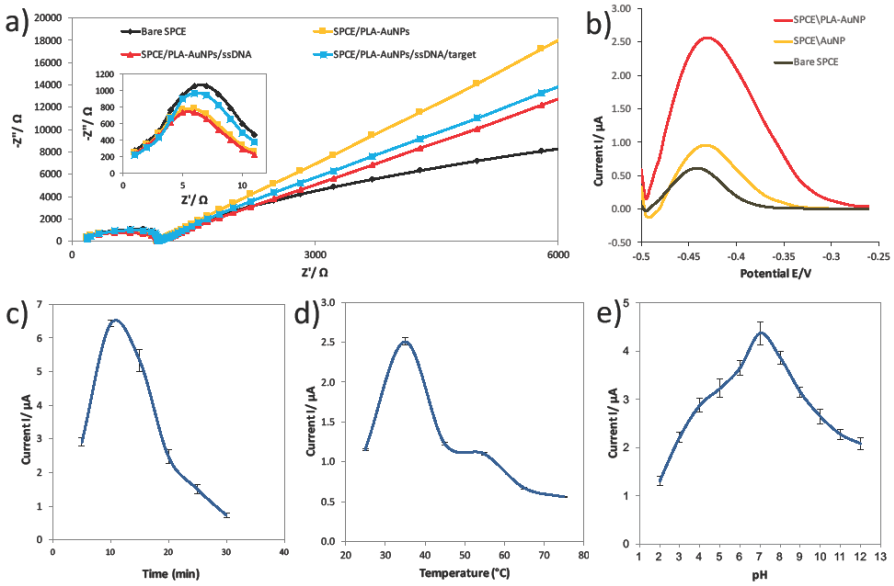
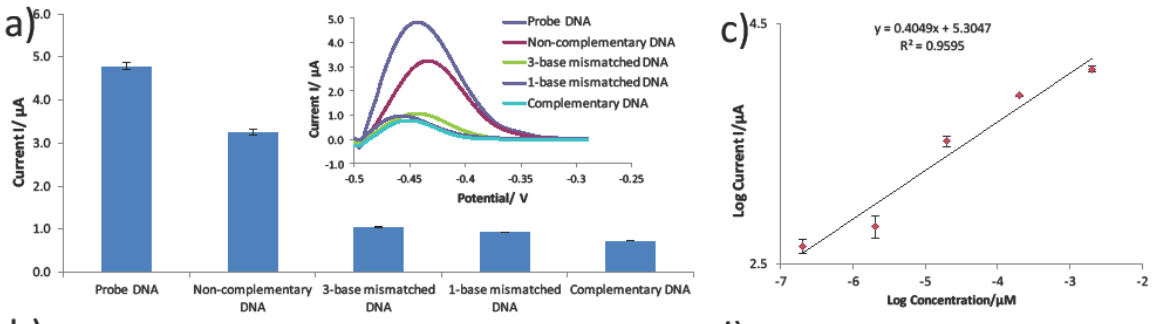
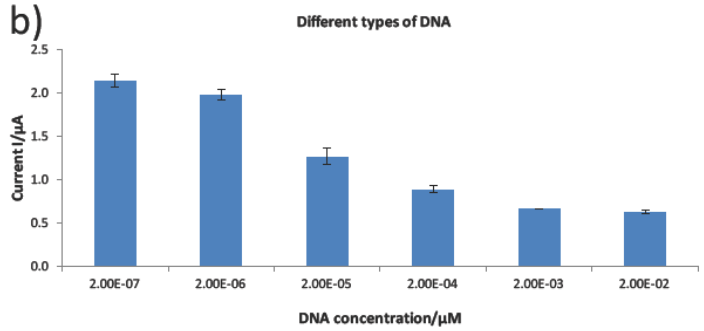
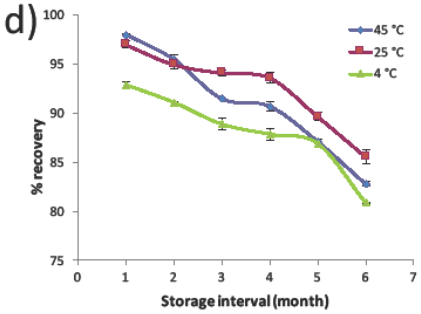
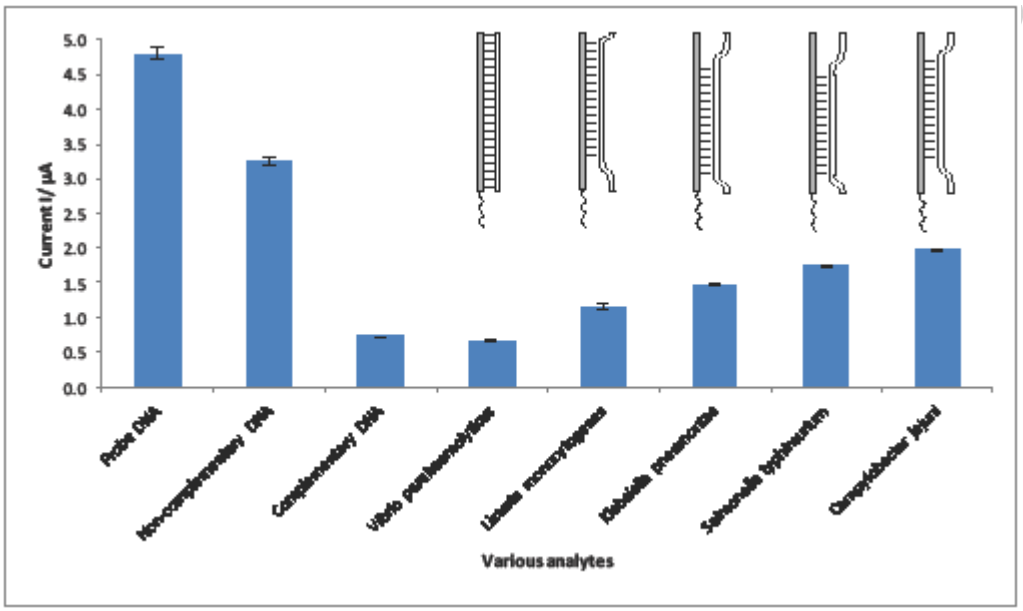

a)
Bare SPCE
SPCE/PLA-AuNPs
SPCE/PLA-AuNPs/ssDNA
SPCE/PLA-AuNPs/ssDNA/target
-Z''/ Ω
Z'/ Ω
b)
SPCE\PLA-AuNP
SPCE\AuNP
Bare SPCE
Current i/ µA
Potential E/V
c)
Current i/ µA
Time (min)
d)
Current i/ µA
Temperature (°C)
e)
Current i/ µA
pH


a)
Current I/ μA
Probe DNA
Non-complementary DNA
3-base mismatched DNA
1-base mismatched DNA
Complementary DNA
Potential/ V
Different types of DNA

c)
y = 0.4049x + 5.3047
R² = 0.9595
Log Current I/μA
Log Concentration/μM


b)
Current I/μA
2.00E-07
2.00E-06
2.00E-05
2.00E-04
2.00E-03
2.00E-02
DNA concentration/μM


d)
45 °C
25 °C
4 °C
% recovery
Storage interval (month)


Current I/ μA
Probe DNA
Non-complementary DNA
Complementary DNA
Various analytes