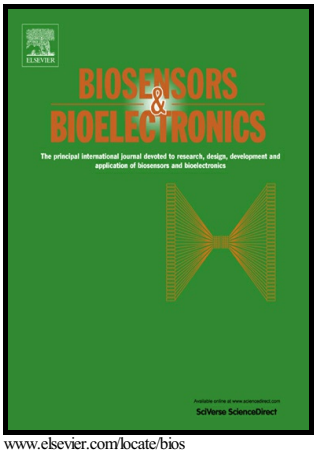

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A Novel Self-powered and Sensitive Label-free DNA Biosensor in Microbial Fuel Cell

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Abstract

In this work, a novel self-powered, sensitive, low-cost, and label-free DNA biosensor is reported by applying a two-chambered microbial fuel cell (MFC) as a power supply. A graphite electrode and an Au nanoparticles modified graphite electrode (AuNP/graphite electrode) were used as anode and cathode in the MFC system, respectively. The active biocatalyst in the anodic chamber was a mixed culture of microorganisms. The sensing element of the biosensor was fabricated by the well-known Au-thiol binding the ssDNA probe on the surface of an AuNP/graphite cathode.

Electrons produced by microorganisms were transported from the anode to the cathode through an external circuit, which could be detected by the terminal multi-meter detector. The difference between power densities of the ssDNA probe modified cathode in the absence and presence of complementary sequence served as the detection signal of the DNA hybridization with detection limit of 3.1 nM. Thereafter, this biosensor was employed for diagnosis and determination of complementary sequence in a human serum sample. The hybridization specificity studies further revealed that the developed DNA biosensor could distinguish fully complementary sequences from one-base mismatched and non-complementary sequences.

Keywords: DNA biosensor; Self-powered; Microbial fuel cell; Au nanoparticles; Cathode.

1. Introduction

DNA biosensors, as detectors of nucleic acid sequences, have attracted an enormous attention over the last three decades with a vast area of applications such as gene analysis, clinical diagnostics, or even forensic applications (Du et al., 2009; Yang et al., 1997). Several techniques have been developed for the sequencing and specific DNA detection including electrochemistry, optical, micro gravimetric quartz-crystal-microbalance methods, and etc (Piunno et al., 1994; Willner et al., 2002; Zhou et al., 2001). Among these methods, electrochemical techniques outperform other detection techniques in terms of simplicity, high sensitivity, lower operating

costs, and compact instrumentation compatible with portable devices (Zhou and Dong, 2011).

New kinds of DNA detection devices have been presented as self-powered biosensors. They have become one of the most widely investigated devices, because of their ability of specific-sequencing without external power source, easy fabrication, and minimizing the scale that directly contributes to the miniaturization of detection devices (Katz et al., 2001; Zhang et al., 2012). As a new effective kind of energy conversion technology, biofuel cells (BFCs) utilize enzymes or microorganisms to catalyze the chemical energy into electrochemical energy (Wang et al., 2014). Until now, many kinds of enzymatic BFCs based self-powered platforms have been developed for chemical and biological sensing, including the detection of glucose (Katz et al., 2001), acetaldehyde (Zhang et al., 2012) and L-Cycteine (Hou et al., 2015).

Despite inherent selectivity of enzymes, for promoting the selective detection, the use of enzymes is limited by their low electron transfer rate, poor enzyme stability, and enzyme loading in BFC technology. On the other hands, there are critical issues regarding high cost of extraction, separation, and purification of enzymes (Zhou and Dong, 2011).

Microbial fuel cells (MFCs) as bio-electrochemical systems represent a new promising source and power technology for the sustainable production of green energy (Ghasemi et al., 2013; Pant et al., 2010). In MFCs, microorganisms as biocatalysts eliminate the requirements for the isolation of individual enzymes, and allow active biomaterials under their natural

environment conditions to convert the chemical energy of organic and inorganic materials in wastewater to electrical energy (Qiao et al., 2007). As a continuous, portability, long life, and safe power source, the MFC technology has been widely used as energy source in electronic devices, implantable medical devices, and biosensors for measuring biochemical oxygen demand (Dai and Choi, 2013).

Herein, we utilized a two-chambered MFC system as a power supply to construct an analytical device for monitoring and characterization of the DNA immobilization and hybridization events. A short sequence of p53 gene was used as ssDNA probe to immobilize on the surface of an AuNP/graphite cathode in the presence of a pH 7.00 phosphate buffer as an electrolyte. P53 is the most important classical type tumor suppressor gene that can mutate in many human cancers (Hong et al., 2014). The ssDNA probe immobilization and hybridization were characterized by the output power of MFC system. Furthermore, the present approach manifests the excellent discrimination of complementary target DNA sequence from one-base mismatched and non-complementary sequences. In the end, the detection limit was calculated to determine the complementary target DNA in human serum sample supported with promising and acceptable results.

2. Experimental

2.1. Reagents and materials

Analytical reagent grade 6-mercapto-1-hexanol (MCH), $K_3[Fe(CN)_6]$, and $K_4[Fe(CN)_6]$ were purchased from Sigma Aldrich. $HAuCl_4*3H_2O$ and tri-sodium citrate dehydrate were purchased from Merck. The synthetic oligonucleotides were obtained from MWG-Biotech Company, and their base sequences are as follow:

ssDNA Probe: 5'-HS-$(CH_2)_6$- TGG GGA TGG AGA ACT-3'

Complementary DNA: 5'-AGT TCT CCA TCC CCA-3'

Non-complementary DNA: 5'-GTT ACT GTT GTA GAT ACT-3'

One-base mismatched DNA: 5'-AGT TCT CCT TCC CCA-3'

The preparation of oligonucleotides stock solutions (10^{-4} M) were carried out using TE buffer solution (10 mM Tris-HCl, 1.0 mM EDTA, pH 8.00).

2.2. Apparatus and calculation

Electrochemical experiments were performed using an AUTOLAB PGSTAT 30 electrochemical analysis system and GPES 4.9 software package (Eco Chemie, Netherlands). The utilized three-electrode system was composed of graphite (modified or unmodified) electrode as the working electrode, an Ag │ AgCl │ KCl (3 M) as the reference electrode, and a Pt wire as the auxiliary electrode. Field emission scanning electronic microscope (FESEM, VEGA2−TESCAN) was used to study the surface and morphological characteristics of the synthesized AuNP/graphite electrode. The voltage was continuously measured by a digital multimeter.

The current and the power produced by the MFC system can be calculated as our previous work (Ghasemi et al., 2013).

2.3. Procedure

2.3.1. The MFC set-up

The laboratory-scale MFC system was set up by connecting two 750 mL Plexiglas chambers as anodic and cathodic compartments with an operating volume of 600 mL. The proton exchange membrane (PEM; Nafion 117, 9.0 cm^2 in total area, Sigma Aldrich, USA) was used to separate the anodic chamber from the cathodic chamber. The cathode consisted of a graphite electrode (0.22 cm^2) coated with synthesized AuNPs. An unmodified graphite plate (12 cm^2) was used as the anode. Both cathode and anode were parallel to the PEM, and fitted by a copper wire to the external circuit.

2.3.2. MFC operation

The anodic chamber was filled with the domestic sludge collected from the anaerobic process tank of the waste water treatment center of Ghaemshahr in the north of Iran. In addition, glucose was used as a carbon source for microorganisms in the domestic sludge. The dissolved oxygen was removed from the anodic chamber by nitrogen gas purging for 10 min. A pH 7.00 phosphate buffer was used as an electrolyte in the cathodic chamber. The prepared MFC system operated at room temperature and neutral pH in both anodic and cathodic compartments.

2.3.3. Preparation of the AuNP/graphite electrode

Spherical AuNPs were synthesized according to our previous work (Asghary et al., 2015a). The graphite electrode surface was mechanically polished on a soft polishing paper, and was washed with distilled water prior to each use. Then, AuNP/graphite electrode was prepared by applying a 12 µL of the AuNPs suspension on the graphite electrode surface and then left to dry. Fig. S1 displays the resulting field emission scanning electron micrograph of the AuNP/graphite electrode. Shiny dots in Fig. S1 represent AuNPs deposited clearly, indicating successful surface modification of the graphite electrode. Thereafter, it was used as the substrate for immobilization of ssDNA probe and DNA hybridization in the next step.

Fig. S1

2.3.4. DNA immobilization and hybridization

A self-assembled monolayer (SAM) of the thiolated ssDNA probe was formed after applying a 12 µL of the ssDNA probe solution (1.0 µM) on an AuNP/graphite electrode surface and left to dry in a wet chamber over night (Hamidi-Asl et al., 2013). Next, the electrode was incubated in 2.0 mM MCH at room temperature for 60 min to prevent any non-specific probe adsorption. The electrode was repeatedly rinsed with distilled water prior to subsequent use. The ssDNA probe modified AuNP/graphite electrode then

incubated in a DNA target solution at room temperature for 1 hour to achieve a double stranded DNA modified electrode (Liu et al., 2010).

3. Results and Discussion

3.1. Electrochemical characterization of the AuNP/graphite electrode

Electrochemical impedance spectroscopy (EIS) is a highly efficient tool to monitor interface properties of the modified electrode surface (Bonanni et al., 2006; Xu et al., 2004). The measured EIS results (offered in form of a Nyquist plot) of different modified electrodes in 10.0 mM $[Fe(CN)_6]^{3-/4-}$ solution containing 0.1 M KCl were demonstrated in Fig. S2. Curve (a) in this figure depicted the Nyquist diagram of $[Fe(CN)_6]^{3-/4-}$ redox couple at surface of the bare graphite electrode with an electron-transfer resistance of about 723 Ω. After modification of the surface of graphite electrode with AuNPs (AuNP/graphite electrode), the impedance spectrum exhibited a 51% decrease in R_{ct} (curve b) compared with that at the bare graphite electrode (curve a). This observation proved that AuNPs led to increased conductivity and surface area of the modified graphite electrode. After immobilization of 1.0 µM ssDNA probe on the surface of AuNP/graphite electrode, semicircle portion of the impedance spectrum increased to R_{ct} = 2037 Ω (curve c). Therewith, the interfacial charge transfer resistance value increased obviously after hybridization of ssDNA probe with the 1.0 µM complementary target DNA sequence (curve d), indicating the negatively charged phosphate skeleton of the adsorbed DNA can repel the redox couple and prevent the electron transfer onto the surface of this electrode.

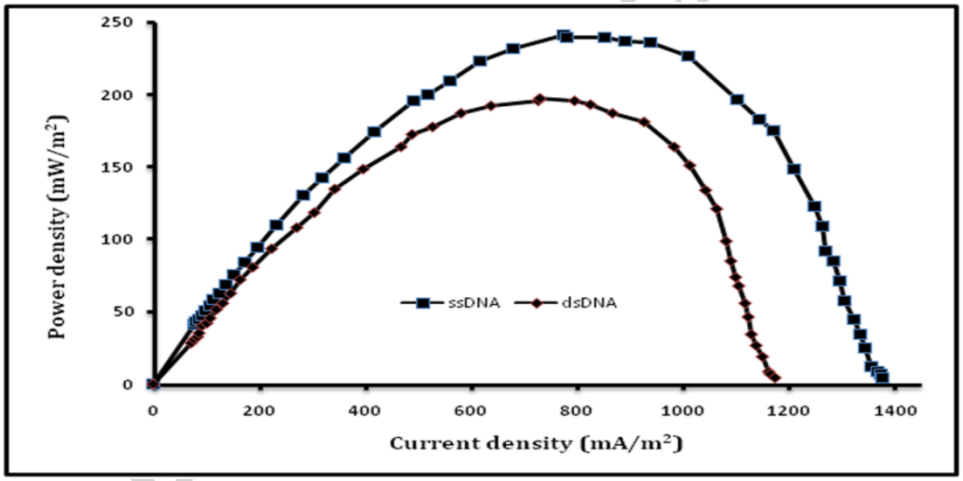
Fig. S2

3.2. Sensitive detection of DNA hybridization in the two-chambered MFC system

The detection of DNA hybridization was carried out by applying the MFC as a new power technology. The two-chambered MFC system operated in a fed-bath mode. The produced power density curve of the MFC system was recorded after 24 hours operation and in the steady state condition at room temperature. To demonstrate the ssDNA probe immobilization and hybridization with complementary target DNA on the surface of an AuNP/graphite cathode, the variation of power density curves vs. current density was obtained in the cathodic chamber. As can be observed in Fig. 1, the maximum power density of 241.55 mW/m^2 was achieved by 1.0 µM ssDNA probe modified AuNP/graphite electrode at a current density of 851.69 mA/m^2. While, after the hybridization of ssDNA probe on the surface of AuNP/graphite electrode with 1.0 µM complementary target DNA, the maximum power density decreased and reached to 190.36 mW/m^2 at a current density of 730.76 mA/m^2. Thus, the obtained power density of the MFC system with complementary DNA hybrid was less than that of with ssDNA. The reason can be explained according to the result of EIS spectra in the previous section, where the DNA double helix molecule with low conductivity acted as a definite kinetic barrier for the electron transfer. This revealed that the ssDNA probe immobilization and

hybridization were effectively detected by the MFC system on the surface of AuNP/graphite electrode.

Fig. 1. Variation of power density as a function of current density of 1.0 µM ssDNA probe modified AuNP/graphite electrode and after hybridization with 1.0 µM complementary target DNA in the presence of a pH 7.00 phosphate buffer as an electrolyte under the steady state condition.



The diagnostic performance of the proposed DNA biosensor is depicted in Fig. 2. The ssDNA probe concentration is constant (1.0 µM), while the complementary target DNA concentration increases from 0.0 to 5.0 µM. Fig. 2 illustrates the power change of ssDNA probe modified AuNP/graphite electrode after hybridization with different concentrations of complementary target DNA in which ΔP (the difference between power

densities of the ssDNA probe modified AuNP/graphite electrode in the absence and presence of complementary target DNA) is enhanced by increasing the target DNA concentration. A detection limit (based on a signal-to-noise ratio of 3) of 3.1 nM is estimated using the plot of ΔP versus the complementary target DNA concentration.

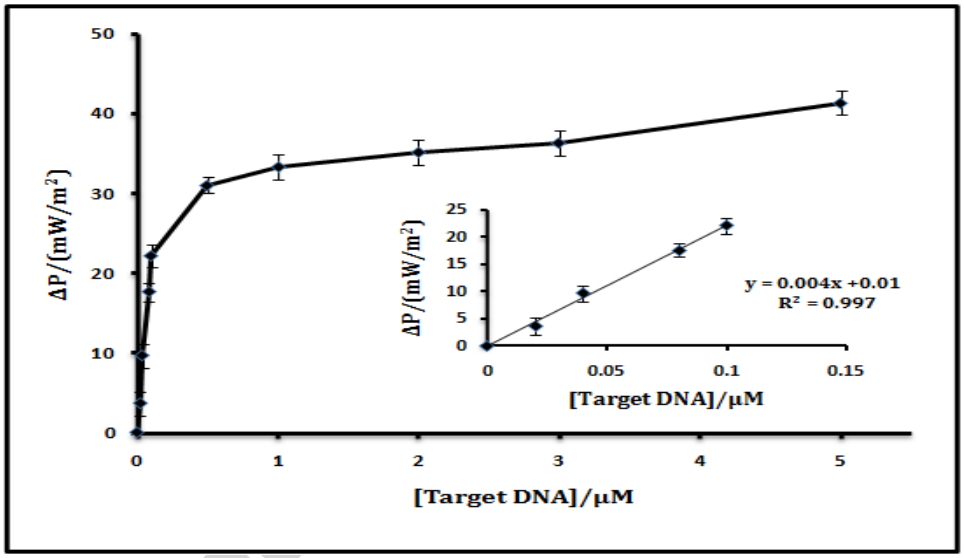


Fig. 2. Plot of ΔP (the difference between the power change of the ssDNA probe modified AuNP/graphite electrode in the absence and presence of complementary target DNA) vs. complementary target DNA concentrations (The ssDNA probe concentration is constant (1.0 µM), while the complementary target DNA concentration increases from 0.0 to 5.0 µM). Inset: related calibration graph at concentration range 0.0-0.1 µM for complementary target DNA.

3.3. Specificity and stability of the proposed DNA biosensor

The specificity of the proposed DNA biosensor was investigated applying different kinds of target oligonucleotides such as complementary, one-base mismatched and non-complementary target DNA sequences with 10^{-8} M concentration. As can be seen in Fig. S3, a high power difference (ΔP) was achieved after hybridization with complementary target DNA i.e. significantly larger than that of one-base mismatched target DNA under the same condition. However, the non-complementary target sequence depicted the same value as ssDNA probe. To further study the specificity of the DNA biosensor via hybridization to the target DNA sequence, a mixed solution containing both complementary and non-complementary target DNA sequences was used. As depicted in Fig. S3, the power difference was partially decreased after hybridization with a complementary sequence in the binary solution of target and non-complementary DNA sequences. This is probably because partial hybridization happened between the immobilized ssDNA probe and non-complementary DNA in the mixture solution. However, the interaction between non-complementary and target sequences has a smaller effect on the hybridization event between ssDNA probe and complementary target sequence (Asghary et al., 2015a; Asghary et al., 2015b). These results revealed that only complementary target sequence formed an effective double helix with the ssDNA probe. In addition, this biosensor could be operated at room temperature for 60 days

by feeding microorganisms with glucose in fed-batch mode (Han et al., 2010). After 60 days operation, there was no apparent change in power generation. These results revealed that the proposed biosensor had a significant stability.

Fig. S3

3.4. Analytical application of the proposed DNA biosensor

The applicability and sensitivity of the proposed DNA biosensor for detection of the complementary target DNA sequence in real-life samples were evaluated using the MFC system in the steady state condition. The spiked human serum solutions contained different concentrations of complementary target DNA sequences. The detection results obtained from three serum solutions are reported in Table 1. The low values of relative standard deviation (RSD) for three replicate measurements (n=3) proved that the proposed biosensor could be potentially used as a sensitive and precise device for diagnosis applications with acceptable reproducibility.

Table 1. Determination of complementary target DNA sequence in spiked human serum samples**.**

Sample	Added[(a)]	Founded[(b)]	RSD[(c)]	Recovery[(d)] %
1	10 nM	9.5 nM	3.73	95
2	60 nM	61 nM	4.21	101.6
3	100 nM	98.81 nM	3.98	98.81

(a) Added means the values that we added into the human serum sample

(b) Founded means the amount of complementary target DNA achieved according to regression equation from Fig. 2.

(c) Relative standard deviation (RSD) after three measurements (n=3).
(d) Recovery of measurements are calculated from the ratio of [Founded]/[Added].

A summary and a comparison of our proposed self-powered DNA biosensor with a BFC based self-powered platform for other chemical and biological sensing have been depicted in Table S1.

Table S1

4. Conclusion

In the present study, we have demonstrated the construction of a truly low cost, easy-to-use, rapid, reproducible, and portable self-powered DNA biosensor applying a MFC system as a core portion and power supply to identify the genetic defects. The power changes before and after hybridization of the ssDNA probe with complementary sequence were sensitively detected by a digital multimeter. Moreover, this biosensor exhibits a successful performance in detection and determination of the complementary sequence in spiked human serum sample and the experimental results showed high sensitivity, significant stability and acceptable reproducibility. Therefore, the developed biosensor could be used as a promising devise for diagnosis application in the future.

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Highlights

- We developed a novel self-powered and truly low-cost DNA biosensor by MFC system.
- The biosensor exhibited high sensitivity to detect target DNA in serum sample.
- The detection limit of 3.1 nM was calculated for the biosensor.
- The biosensor could be used as sensitive and precise device for diagnosis applications.
- There is no publishing about using of MFC system in construction of DNA biosensors.