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Electrochemical nanoporous alumina membrane-based label-free DNA biosensor for the detection of *Legionella sp*

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ABSTRACT

An electrochemical nanoporous alumina membrane-based label free DNA biosensor is developed using 5′-aminated DNA probes immobilized into the nanochannels of alumina. Alumina nanoporous membrane-like structure is carved over platinum wire electrode of 76 μm diameter dimension by electrochemical anodization. The hybridization of complementary target DNA with probe DNA molecules attached inside the nanochannels influences the pore size and ionic conductivity. Electrochemical biosensing signal is derived from only redox species $Fe(CN)_6^{4-}$ across single wire Pt electrode. The biosensors sensing mechanism relies on the monitoring of electrode's Faradaic current response toward redox species, $Fe(CN)_6^{4-}$, which is sensitive toward the hybridization of complementary target with probe DNA immobilized into the alumina nanochannels. The biosensor demonstrates wide linear range over 7 orders of magnitude with ultrasensitive detection limit 3.1×10^{-13} M for the quantification of ss 21mer DNA sequence and selectively differentiates the complementary sequence from target sequences with single base mismatch (MM1) and triple bases mismatch (MM3) of different strain of *Legionella sp*. Its applicability is also challenged against real time *Legionella pneumophila* genomic DNA sample derived from the asymmetric PCR method.

1. Introduction

Among various pathogenic bacteria, *Legionella pneumophilla* is a fatal pathogen which causes a serious form of pneumonia known as Legionnaires' disease [1,2]. The bacteria is commonly found in cooling water towers and can be transmitted air-borne in poorly ventilated rooms. Rapid diagnosis of this disease is crucial for efficient treatment and patient survival. Hence it is essential to achieve early detection of *Legionella* species to prevent legionellosis and monitor epidemic outbreaks. Several diagnostic tools have been developed for the detection of *L. pneumophila* till date. Current methods are based on culture techniques, but these are time consuming and require at least 3–10 days in sampling. Additional problems with culture detection include low sensitivity, microbial contamination inhibiting *Legionella* growth, and the potential presence of viable but nonculturable bacteria (VBNC) [3,4]. Methods based on direct detection, combining immunofluorescent labeling (IF) [5] or fluorescent in situ hybridization (FISH) [6,7] with detection by epifluorescence microscopy or flow cytometry [8] allow a more rapid detection of *Legionella* cells and avoid most of the problems encountered with culture.

Alternatively, PCR-based assays have been developed for *Legionella* but remain limited mainly because of (i) the potential presence of PCR inhibitors, (ii) the lack of information on the viability of cells, and (iii) the low sensitivity for the quantification of cells direct fluorescent antibody (DFA) stain of sputum [9,10]. An indirect immunofluorescence assay (IFA) on serum, and a urine *Legionella* antigen (ULA) assay are also used to detect *Legionella sp* [11–13]. However, the above methods are tedious and time-consuming, expensive for mass screening, and characterized by low levels of sensitivity and specificity. More recently, diagnostic polymerase chain reaction (PCR) and real-time PCR assays based on nucleic acid amplification have been used [14,15]. However, the analysis still takes several hours, and this approach requires highly meticulous handling and sophisticated instrumentation to conduct the assays. These characteristics are potential drawbacks in the design of field-based portable devices for the diagnosis of *L. pneumonia* in large populations. Zelada-Guillen et al. (2010) have used nanotube modified glassy carbon electrode to detect pathogenic microbes [16]. Nanoporous membrane based biosensors have been used to detect small molecules proteins [17], cells [18], virus [19], metal ions [20] and DNA [21,22]. Recently, interesting solid state bio-functionalized nanopores biosensor have been developed to detect complementary target molecules present in solution, using electrophoretically drawn movement through the nanometric channel by Mussi et al. [21,23].

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Herein we use electrochemical methods to detect target DNA analyte of *Legionella sp* because of simple instrumentation, low cost, portability, fast response time and low detection limit. However, the detection limit and sensitivity of electrochemical biosensors are considerably poorer than fluorescence probes, which can give high intensity signal arising from rapid turnover of excited and ground states of fluorophore label and a continuous excitation source, thus achieve high signal-to-noise ratio. Electrochemical detection methods offer high specificity, sensitivity and onsite analysis applicability with potential for developing molecular sensing devices [24–27]. Electrochemical detection involves redox species, metal ions enzymes and intercalators physically or covalently adsorbed on probe or target. In electrochemical biosensors, specific biomarker molecules are coupled to electrochemical platforms and selective binding of target analytes are translated into electrochemical signal. Binding of target analyte to probe DNA attached on electrode changes the electrochemical faradic current, capacitance, resistance and electrical impedance property of interface between electrodic surface and electrolyte. In our previous work we have shown detection of *Legionella sp* with probe label with redox sp ferrocene. Herein, we demonstrate development of alumina membrane based label free electrochemical DNA biosensor for 21-mer analyte DNA sequence detection of *L. pneumophila*.

Electrochemical anodization of aluminum results in a nanoporous multi-channel alumina structure with pore size range from 10 to 150 nm and density of about 1×10^{10} pores cm^{-2} [28]. This anodization technique is comparatively easier than conventional lithographic methods. We attach 32-mer probe DNA sequences covalently into the channels of nanoporous alumina membrane based electrode. Nanoporous alumina electrodic structure shows unique property of high aspect ratio and high surface area to incorporate specific probe molecules. Binding of target complementary DNA to probe inside nanochannels causes changes in ionic conductivity of redox species $Fe(CN)_6^{4-}$ through it due to blocking of the pores. Ionic conductivity changes through alumina nanopores are translated into electrochemical signal using differential pulse voltammetric (DPV) technique. The DPV oxidative peak current of $Fe(CN)_6^{4-}$ successively drops with increase in target complementary DNA concentration which is consistent with increase in resistance value obtained from electrochemical impedance spectroscopy (EIS).

2. Materials and methods

2.1. Reagents

DNA probe sequence of *L. pneumophila* (5′–$NH_2(CH_2)_6$ TCGA **TAC TCT CCC CGC CCC TT T TGT** ATCGACG- 3′) complementary target sequence (5′–ACA AAA GGG GCG GGG AGA GTA-3′), single nucleotide mismatch target sequence (5′–ACA AAA GG**A**GCG GGG AGA GTA-3′), three nucleotide mismatch target sequence (5′–**G**CA AAA GGG GCG GGG AGA G**GG**-3′), potassium hexacyanoferrate (II) trihydrate, potassium hexacyanoferrate (III), chromic acid, phosphoric acid (85%), 3-aminopropyltrimethoxysilane (APS), glutaraldehyde (25 wt% solution in water), propylamine, sodium chloride, platinum wire 99.99% (0.076 diameter), 1.0 M tris (2-carboxy-ethyl) phosphine hydrochloride (TRIS buffer) of pH 7.0, were obtained from Sigma Aldrich. All target analyte DNA solutions were prepared using 1.0 M TRIS buffer pH 7.0. 1 M and 1 X phosphate buffer saline (PBS) solution (pH 7.2) was obtained from 1st Base. Alumina powder (1 μm and 0.3 μm) were purchased from Allied High Tech Products, Inc. Epoxy structural adhesive DP 760 was obtained from 3 M Technologies (S) Pte Ltd. Alumina target 99.999% purity was obtained from Optoelectron Technologies. All reagents were used as received, unless otherwise stated.

2.2. Procedure for analyses of DNA targets

The nanoporous alumina based DNA biosensor was thermostated in the complementary target solution at 45 °C. The biosensor was subsequently rinsed with ultrapure water to remove any unhybridized target, followed by electrochemical measurements at room temperature. Electrochemical measurements were performed using CV, DPV and EIS techniques, DPV signal was recorded of bare alumina electrode followed by its successive modification with probe DNA and aftermath hybridization with complementary target to investigate electrochemical response of the biosensor. To avoid false positive signal response owing to loss of DNA probes from the biosensor during the heating cycle, the biosensor was subjected to preconditioning step of a continuous series of heating cycles in the absence of complementary DNA target. Therefore, the biosensor preparation procedure comprises a final pre-conditioning step of three consecutive heating cycles of 75 °C, 30 min each, to ensure reproducible biosensor responses before use. To achieve selective discrimination of target DNA analyte with one and three base mismatch, the biosensor was thermostated at 57 °C for 30 min followed by taking out biosensor from thermostat solution and allowing it to cool down up to room temperature, rinsing in ultrapure water followed by electrochemical measurements. Melting/ hybridization temperature of target analyte DNA of one and three base mismatch (44 °C and 56 °C respectively) was suitably exploited in thermostatic incubation to obtain selective discrimination against complementary target. Nanoporous alumina membrane based biosensor was also tested against 157 base genomic sequence of *L. pneumophila* real time sample derived from the asymmetric PCR method. PCR products were subsequently diluted by 10-fold up to three serial dilutions for detection by the biosensor.

2.3. Construction of nanoporous alumina membrane-based DNA biosensor

The fabrication design and operating principle of nanoporous alumina membrane based DNA biosensor are shown in Fig. 1. Homemade electrodes were fabricated using chemical resistant epoxy resin (RS Components Pte Ltd.), micropipette tips and 99.99% platinum wire (76 μm diameter, Sigma Aldrich). The platinum wire was aligned in the center of the micropipette tips and sealed within epoxy resin. The platinum wire was subsequently soldered to a copper wire and the connection was sealed with epoxy resin. The fabricated platinum wire electrodes were polished with 1.0 μm and 0.3 μm diameter alumina slurry and sonicated in ultrapure water (with resistivity of more than 18 Ω). Sub-micrometer thick aluminum films were sputter coated over the platinum electrodes using 99.999% purity aluminum target, Denton discovery® 18 Sputtering System and sputtering power of 100 W in an atmosphere of research-grade Ar at 5×10^{-3} Torr. Anodization of aluminum coated electrodes was conducted using a previously described method of surface contact anodization [29].

5′-aminated 32-mer DNA probes were covalently attached onto nanoporous alumina using glutaraldyhyde cross linking [30]. The nanoporous alumina electrodes were immersed in 5% APS solution for an hour and dried in vacuum oven for 30 min at 45 °C after thorough washing with acetone and drying with argon. APS activated naonoporous alumina electrodes were immersed in glutaraldehyde for 12 h, followed by thorough washing with ultrapure water and drying with argon. ~50 μL of 100 μM of 5′ aminated DNA probe solution was added onto the surface and

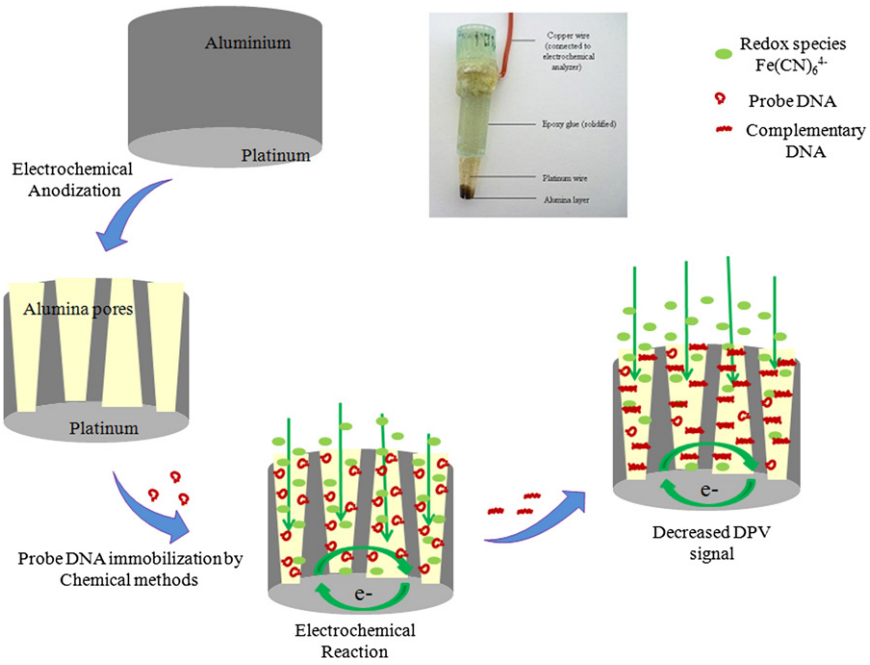

Fig. 1. Scheme of construction and operation for nanoporous alumina membrane based. Electrochemical label free DNA biosensor.

kept at high humidity overnight. The electrodes were subsequently rinsed with 1 M NaCl to remove any non-specific adsorbed DNA and dried in argon. Few drops of 10^{-6} M of propylamine was added onto the nanoporous alumina electrodes and left for 6 h to neutralize excess glutaraldehyde and facilitating efficient hybridization with complementary target during thermostatic incubation, followed by thorough washing of the electrodes was performed using ultrapure water and then dried in argon.

2.4. Electrochemical measurements

Electrochemical behaviors of the alumina modified electrodes were investigated using cyclic voltammetry (CV) and differential pulsed voltammetry (DPV) techniques (CHI 750 potentiostat/galvanostat, data acquisition software) in the presence of 1.0 mM $Fe(CN)_6^{4-}$ in 1 M 1X phosphate buffer solution, pH 7.2 using a three electrode system. The nanoporous alumina pipette electrode biosensor was used as working electrode and all potentials and currents were measured with respect to the Ag/AgCl (1.0 M KCl) reference electrode and Pt gauze counter electrode under ambient conditions. Differential pulse voltammetry was carried out using 50 ms pulse width, 50 mV pulse height, pulse period of 200 ms and potential increment of 1 mV and CV was recorded in potential window of −0.1 to 0.7 V with scan rate of 50 mV s^{-1}. Electrochemical Impedance Spectroscopy (Auto lab with Nova software) measurements were performed in 1X phosphate buffer saline (PBS) solution (pH 7.2) in the presence of $Fe(CN)_6^{3-/4-}$ (5 mM 1:1) at 0.22 V.

3. Results and discussion

3.1. Electrochemical characterization of nanoporous alumina membrane based DNA biosensor from cyclic voltammetry (CV)

Nanoporous alumina membrane structure resulted after electrochemical anodization over Pt single wire electrode (diameter 76 μm) acts as suitable substrate for single stranded DNA probe

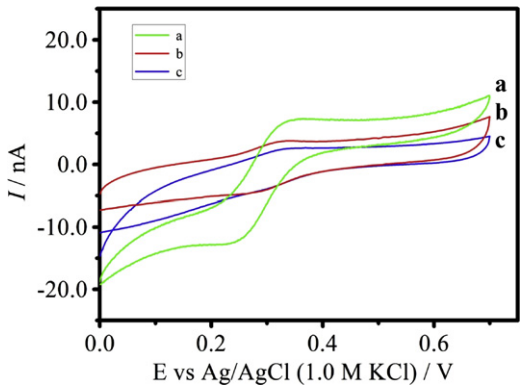

Fig. 2. Cyclic voltammograms of bare nanoporous alumina membrane based electrode (a), modified electrode with probe (b) and treatment of modified electrode with complementary target (c) in the presence of redox species (1 mM $Fe(CN)_6^{4-}$ in 1X PBS).

immobilization. Fig. 2 shows cyclic voltammograms of bare nanoporous alumina membrane electrode, its subsequent modification with probe molecules and complementary hybridization with probe into the nanochannels in the presence of redox species (1 mM $Fe(CN)_6^{4-}$ in 1X PBS). As can be seen peak current in CV decreases from bare electrode to modified electrode with probe DNA due to blocking of redox species along the wall of nanochannels. Thus mass transfer of redox species are restricted to access Pt electrode for electrochemical communication as faradic current in cyclic voltammogram depends on the concentration of redox species. Treatment of modified electrode with complementary target DNA leads to further decrease in peak current of cyclic voltammogram due to further increase in blocking the wall of nanochannels after probe complementary hybridization. Thus nanochannels structure over Pt electrode are suitably exploited to detect label free DNA in contrast to label DNA detection where additional tedious synthetic steps are required to label either probe or target analyte DNA. However because of large capacitive currents in potential cycling of nanoporous membrane electrode limits the extraction of reproducible peak currents with different

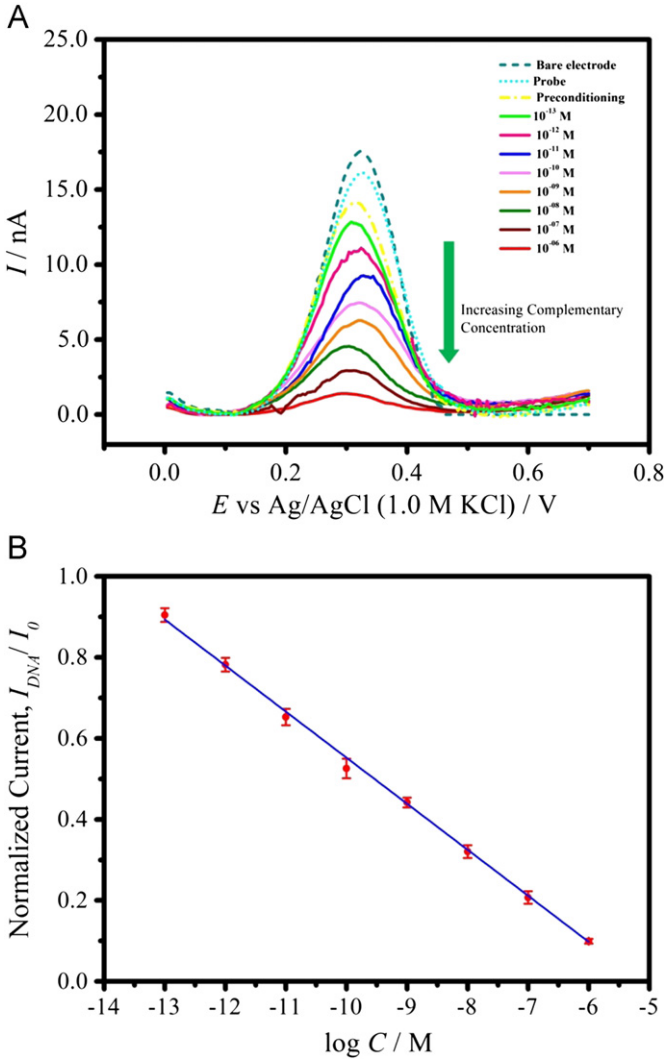

Fig. 3. (A) Differential pulse voltammetry current signal response of bare electrode toward increasing concentration of complementary target from 10^{-13} to 10^{-6} M. DPV currents were offset to 0 μA to allow comparison of results and all measuring solutions contain 1X, pH 7.2 PBS electrolyte solution. (B) Averaged normalized current signal response best fitted linearly with log *C* of complementary solutions. Error bars and points represent average standard deviations derived from single biosensor with three consecutive measurements.

concentrations of target analyte and offering challenge for ultrasensitive detection of DNA using cyclic voltammetry.

3.2. Nanoporous alumina membrane based DNA biosensor signal derived from differential pulse voltammetry (DPV)

Fig. 3(A) shows the DPV peak currents of bare nanoporous alumina electrode, modified electrode with probe DNA and different concentration of complementary analyte solution. Differential pulse voltammetry technique excludes large capacitive currents in comparison to nonpulsed voltammetry technique. Thus DPV technique facilitates signal acquisition corresponding to even at lower concentration of target analyte. Successive drop in differential oxidative peak currents are observed with increase in concentration of complementary analyte over wide concentration range. This drop in oxidative peak current is attributed to complementary and probe hybridization inside the alumina nanochannels. These nanochannels are of subnanometer dimension and diffusion of redox species from bulk solution to sensing Pt single wire electrode through these nanochannels are influenced in presence of complementary target. Thus in the presence of complementary target, diffusion of redox species are blocked along the nanochannels and hybridization events are sensed as drop in oxidative peak current of redox species across Pt electrode.

Fig. 4 shows the impedance spectra Nyquist Plot of bare nanoporous alumina membrane electrode, modified electrode with probe and modified electrode treated with complementary analyte solutions of 10^{-8} and 10^{-6} M. EIS is used to investigate the changes in electrical property associated with nanoporous alumina membrane electrode surface modification and subsequent treatment with complementary solution. Equivalent circuit [(RC)(RC)(QC)] is used to fit the raw data of frequency scan with electrical elements *R* charge transfer resistance, *Q* constant phase element and *C* capacitance to derive quantitative information. The impedance spectrum changes with the function of surface modification of nanoporous alumina membrane electrode. As can be seen from the fitted equivalent Nyquist plot *R* value successively increases 10-fold from bare electrode (11.9 kΩ) to modified electrode (112 kΩ) due to probe immobilization along the nanochannels and restricting access of redox species to single wire Pt electrode. Moreover, treatment of modified nanoporous alumina membrane electrode with increasing concentrations of complementary analyte 10^{-8} and 10^{-6} M, further increases the resistance value from 222 kΩ to 254 kΩ respectively consistent with our observed differential oxidative peak currents response at nanoampere scale as shown in Fig. 3(A).

3.3. Analytical performance

Fig. 3(B) shows the plot of biosensor current signal responses versus the logarithm of complementary ssDNA target concentration in 1 mM $Fe(CN)_6^{4-}$ of supporting electrolyte 1X PBS buffer (pH 7.4). Linear decrease in normalized DPV current signal response against complementary ssDNA target concentration is observed over wide linear range from 10^{-13} to 10^{-6} M (R^2=0.98). The detection limit of electrochemical nanoporous alumina membrane based DNA biosensor is determined from the minimum complementary analyte concentration which gives a signal reduction equivalent to three times the standard deviation of signal in the absence of complementary. The low detection limit of 3.1×10^{-13} M outperforms those using PCR based DNA detection. Moreover, rapid analysis time of ~45 min and fairly low

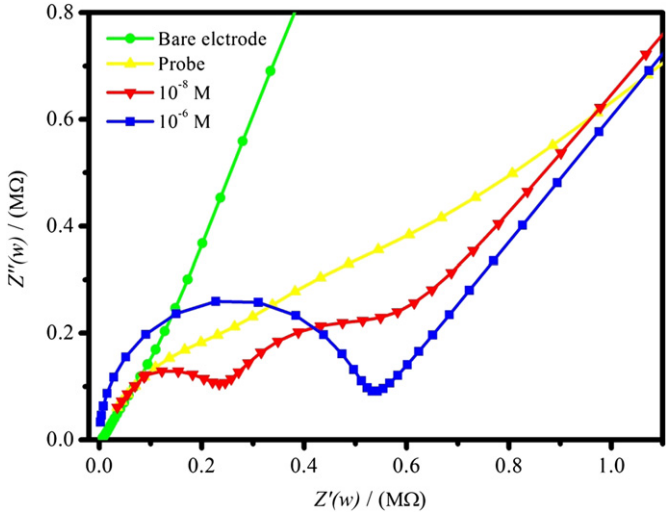

Fig. 4. Impedance spectra Nyquist Plot of bare nanoporous alumina membrane electrode, modified electrode with probe and modified electrode treated with complementary analyte solutions of 10^{-8} and 10^{-6} M.

detection limits of nanoporous alumina membrane based DNA biosensor for a 21-mer DNA sequence of *Legionella sp*, present significant improvement of 6–7 orders over DNA sensor based on colorimetry [31], optical [32] and fluorescence [33] and is comparable to nonPCR based enzyme amplified [34], electrochemically amplified label DNA biosensor [35] and impedimetric biosensor [36].

3.4. Specific response toward target with single base mismatch (MM1) and triple bases mismatch (MM3)

Fig. 5 shows the normalized current signal response of nanoporous alumina based biosensor toward 21-mer complementary target sequence generally found in *Legionella sp* and target analyte with single base mismatch (MM1) and triple bases mismatch (MM3). These MM1 and MM3 target sequences are found in Lens strain of *L. pneumophila* and *A. hydrophila*, respectively. Significant drop down in normalized current signal response in observed in contrast to target sequence with single base mismatch and triple bases mismatch. During analysis of the complementary target and target sequence with single base mismatch (MM1), the biosensor was thermostated at 45 °C, which is fairly lower than melting temperature (T_m 62 °C) of complementary analyte and higher than melting temp (T_m 44 °C) of target sequence with MM1 (melting temperatures were calculated using nearest neighbor thermodynamics based software biomath T_m calculators). Thus complementary target selectively hybridizes with probe immobilized into the alumina nanochannels of biosensor and target sequence remains unhybridized. As two complementary strands of DNA remain hybridized at all temperatures lower than its melting temperature above which both strands unhybridize and melt away. The biosensor was incubated at 58 °C with triple bases mismatch target sequence MM3 (T_m 57 °C), consequently target MM3 does not hybridize with the probe. Therefore target sequences with single base mismatch and triple bases mismatch do not show significant changes in normalized DPV current signal response in contrast to complementary target sequence. In addition, the used biosensor can be regenerated with very good reproducible normalized signal response by incubating in a pH 7.0, 0.5 M Tris buffer for 30 min at 75 °C. It is noteworthy to mention that though the current signal error is relatively small (5.3% error), because of the logarithmic dependence of the concentration range, each measurement gives an error of ca. one order of magnitude in the DNA concentration.

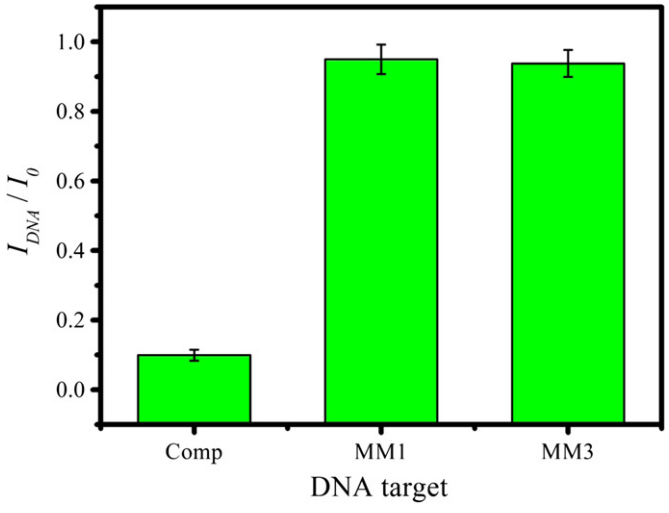

Fig. 5. Changes in normalized differential current signal of the biosensor probe toward 10^{-6} M 21-mer complementary sequence and target analyte DNA sequence with single-base mismatch (MM1) and triple bases mismatch (MM3) respectively. Error bars correspond to standard deviations obtained from 3 consecutive DPV measurements.

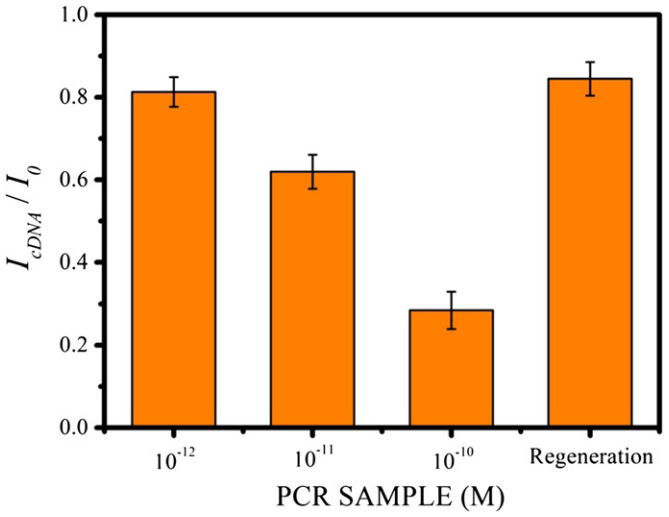

Fig. 6. Normalized differential current signal response of biosensor toward this real time DNA PCR sample of 10^{-12}, 10^{-11}, and 10^{-10} M, derived from *Legionella sp* genomic sequence using the asymmetric PCR method. Error bars correspond to standard deviations obtained from 3 consecutive DPV measurements.

3.5. Detection of Legionella pneumophila genomic DNA

In order to test the applicability of the biosensor in real sample analysis, it is challenged with PCR amplicons for *Legionella* genomic DNA. A 157-bp region between positions 58 and 78 in the genomic DNA (5′–TGAAGTGGTGATTGGAGGTAATTACACAGTAGGTATAGTGG TATTTATTATCCTTGTCGTTATTAACTTTGTTGTAGTGACAAAAGGGGC GGGGAGAGTATCTGAAGTAAGTGCGCGGTTTACTTTGGATGCTTTACC CGGAAAGCAAATGGCTATC- 3′) was selected as the target, with complementary sequence of the probe DNA covalently immobilized into the alumina nanochannels of the biosensor. Fig. 6 shows the biosensor signal response toward serial diluted PCR amplicon samples of the isolated target sequence. Successive dropdown in normalized current signal response of biosensor is observed toward increasing concentration of ssDNA PCR samples of *Legionella sp*, and the biosensor can be regenerated after exposure to the series of diluted PCR amplicon samples using 75 °C, 25–30 min heating cycle. Thus nanoporous alumina membrane based DNA biosensor demonstrates the potential use in detecting genomic sequence of pathogens for environmental monitoring.

4. Conclusions

The electrochemical nanoporous alumina membrane based label free DNA biosensor shows ultrasensitive detection of 21-mer complementary analyte (3.1×10^{-13} M) with wide linear range derived from DPV current signal from 10^{-6} to 10^{-13} M. Sensing of 157 bases long genomic DNA sequence of *Legionella sp* derived from the asymmetric PCR method is also achieved. The biosensor selectively differentiates the complementary sequence from target sequences with single base mismatch (MM1) and triple bases mismatch (MM3) of different strain of *Legionella sp*. The sensitivity of this biosensor outperforms existing label free DNA biosensor. The construction of nanoporous alumina membrane based DNA biosensor is very simple and relatively easier to carve nanoporous structure by electrochemical anodization than conventional lithography e.g. electron beam or focussed ion beam and sample analysis time is around ~45 min. Electrochemical

biosensing signal is derived only from $Fe(CN)_6^{4-}$ across single wire Pt electrode in contrast to label DNA sensor and amplified DNA sensor where redox active or label is attached in the probe or target DNA which requires additional synthetic and purification steps.

Acknowledgments

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References

[1] A.L. Reingold, B.M. Thomason, B.J. Brake, J. Infect. Dis. 149 (1984) 819.
[2] V.L. Yu, J.F. Plouffe, M.C. Pastoris, J.E. Stout, M. Schousboe, A. Widmer, J. Summersgill, T. File, C.M. Heath, D.L. Paterson, A. Chereshsky, J. Infect. Dis. 186 (2002) 127–128.
[3] A.K. Bej, M.H. Mahbubani, R.M. Atlas, Appl. Environ. Microbiol. 57 (1991) 597–600.
[4] F. Joux, P. Lebaron, Microbes Infect. 2 (2000) 1523–1535.
[5] C.J. Palmer, Y.L. Tsai, C. Paszko-Kolva, C. Mayer, L.R. Sangermano, Appl. Environ. Microbiol. 59 (1993) 3618–3624.
[6] S. Buchbinder, K. Trebesius, J. Heesemann, Int. J. Med. Microbiol. 292 (2002) 241–245.
[7] W. Satoh, M. Nakata, H. Yamamoto, T. Ezaki, K. Hiramatsu, Appl. Environ. Microbiol. 68 (2002) 6466–6470.
[8] R.L. Tyndall, R.E. Hand Jr, R.C. Mann, Appl. Environ. Microbiol. 49 (1985) 852–857.
[9] V. Catalan, F. Garcia, C. Moreno, M.J. Vila, D. Apraiz, Res. Microbiol. 148 (1997) 71–78.
[10] N. Wellinghausen, C. Frost, R. Marre, Appl. Environ. Microbiol. 67 (2001) 3985–3993.
[11] B.S. Fields, R.F. Benson, R.E. Besser, Clin. Microbiol. Rev. 15 (2002) 506–526.
[12] G.W. Waterer, V.S. Baselski, R.G. Wunderink, Am. J. Med. 110 (2001) 41–48.
[13] H.W. Wilkinson, A.L. Reingold, B.J. Brake, J. Infect. Dis. 147 (1983) 23–31.
[14] J.L. Cloud, K.C. Carroll, P. Pixton, M. Erali, D.R. Hillyard, J. Clin. Microbiol. 38 (2000) 1709–1712.
[15] K.E. Templeton, S.A. Scheltinga, P. Sillekens, J.W. Crielaard, A.P. Van Dam, H. Goossens, E.C.J. Claas, J. Clin. Microbiol. 41 (2003) 4016–4021.
[16] G.A. Zelada-Guillen, S.V. Bhosale, J. Riu, F.X. Rius, Anal. Chem. 82 (2010) 9254–9260.
[17] M. Kang, L. Trofin, M.O. Mota, C.R. Martin, Anal. Chem. 77 (2005) 6243–6249.
[18] M.S. Cheng, S.H. Lau, V.T. Chow, C.S. Toh, Environ. Sci. Technol. 45 (2011) 6453–6459.
[19] Binh T.T. Nguyen, K. Guiwan, H.S. Lim, A.J.S. Chua, M.M.L. Ng, C.S. Toh, Anal. Chem. 81 (2009) 7226–7234.
[20] S. Zairi, C. Martelet, N. Jaffrezic-Renault, R. Lamartine, R. M'Gaieth, H. Maaref, M. Gamoudi, G. Guillaud, Appl. Surf. Sci. 172 (2001) 225–234.
[21] V. Mussi, P. Fanzio, L. Repetto, G. Firpo, S. Stigliani, G.P. Tonini, U. Valbusa, Biosens. Bioelectron. 29 (2011) 125–131.
[22] P. Takmakov, I. Vlassiouk, S. Smirnov, Analyst 131 (2006) 1248–1253.
[23] V. Mussi, P. Fanzio, L. Repetto, G. Firpo, P. Scaruffi, S. Stigliani, M. Menotta, M. Magnani, G.P. Tonini, U. Valbusa, J. Phys. Condens. Matter 22 (2010) 225–234.
[24] A. Bini, M. Minunni, S. Tombelli, S. Centi, M. Mascini., Anal. Chem. 79 (2007) 3016–3019.
[25] A.J. Bergren, R.L. McCreery, Annu, Rev. Anal. Chem. 4 (2011) 173–195.
[26] C. Tlili, H.K. Youssoufi, L. Ponsonnet, C. Martelet, N.J.J. Renault, Talanta 68 (2005) 131–137.
[27] J. Zhang, S. Dong, J. Lu, A.P.F. Turner, Q. Fan, S. Jia, X. Lu, H. Yang, C. Qiao, H. Zhou, G. He, Anal. Lett. 42 (2009) 2905–2913.
[28] E. Ekanayake, D.M.G. Preethichandra, K. Kaneto, Biosens. Bioelectron. 23 (2007) 107–113.
[29] G. Koh, S. Agarwal, P.S. Cheow, C.S. Toh, Electrochim. Acta 52 (2007) 2815–2821.
[30] I. Vlassiouk, A. Krasnoslobodtsev, S. Smirnov, M. Germann, Langmuir 20 (2004) 9913–9915.
[31] B. Bockisch, T. Grunwald, E. Spillner, R. Bredehorst, Nucleic Acids Res. 33 (2005) e101.
[32] F. Wei, C. Chen, L. Zhai, N. Zhang, S.Z. Xin, J. Am. Chem. Soc. 127 (2005) 5306–5307.
[33] H. Du, M.D. Disney, B.L. Miller, T.D. Krauss, J. Am. Chem. Soc. 125 (2003) 4012–4013.
[34] G. Liu, Y. Wan, V. Gau, J. Zhang, L. Wang, S. Song, C. Fan, J. Am. Chem. Soc. 130 (2008) 6820–6825.
[35] V. Rai, Y.T. Nyine, H.C. Hapuarachchi, H.M. Yap, L.C. Ng, C.S. Toh, Biosens. Bioelectron. 32 (2012) 133–140.
[36] Y.T. Long, C.Z. Li, T.C. Sutherland, H.B. Kraatz, J.S. Lee, Anal. Chem. 76 (2004) 4059–4065.