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Microfluidics and nanoparticles based amperometric biosensor for the detection of cyanobacteria (*Planktothrix agardhii* NIVA-CYA 116) DNA

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ABSTRACT

Some of the cyanobacteria produce protease inhibitor oligopeptides such as cyanopeptolins and cause drinking water contamination; hence, their detection has great importance to monitor the well-being of water sources that is used for human consumption. In the current study, a fast and sensitive nucleic acid biosensor assay has been described where cyanopeptolin coding region of one of the cyanobacteria (*Planktothrix agardhii* NIVA-CYA 116) genome has been used as target for monitoring of the fresh water resources. A biochip that has two sets of Au electrode arrays, each consist of shared reference/counter electrodes and 3 working electrodes has been used for the assay. The biochip has been integrated to a microfluidics system and all steps of the assay have been performed during the reagent flow to achieve fast and sensitive DNA detection. On-line hybridization of the target on to the capture probe immobilized surface resulted in a very short assay duration with respect to the conventional static assays. The binding of the avidin and enzyme modified Au nanoparticles to the biotinylated detection probe and the subsequent injection of the substrate enabled a real-time amperometric measurement with a detection limit of 6×10^{-12} M target DNA (calibration curve $r^2 = 0.98$). The developed assay enables fast and sensitive detection of cyanopeptolin producing cyanobacteria from freshwater samples and hence shows a promising technology for toxic microorganism detection from environmental samples.

1. Introduction

Occasionally, fresh water lakes and water sources may be threatened by cyanobacterial toxic blooms that constitute a potential health risk to human population and the aquatic organisms (Marie et al. 2012). Conventionally, the toxins produced by cyanobacteria can be detected by enzyme-linked immunosorbent assay (ELISA) (Lotierzo et al. 2012), high performance liquid chromatography (HPLC) (Meriluoto et al. 1998) or protein phosphatase assay (Martins et al. 2011). Although the detection of the toxins in a fresh water source is very important, this data does not provide enough information about the presence of the toxin producing cyanobacteria (Johnson and Mutharasan 2013). A fast and sensitive detection of the cyanobacteria would be very important to monitor the well-being of the fresh water sources, before their numbers and the amount of the toxin they produce increase. Analytical or molecular methods such as microscopic counting and identification, or PCR tests have been developed for the detection and quantification of the cyanobacteria (Churro et al. 2012; Pearson and Neilan 2008; Tonk et al. 2005). Biosensors have the potential to provide an alternative to the current nucleic acid based tests if the required sensitivity is reached and the size of the biosensors is miniaturized. In recent years, the research and development on DNA detection biosensors has gained pace due to their extreme potential for a variety of applications, such as diagnostics, toxicology (Humbert et al. 2010), food safety (Kalogianni et al. 2006), environmental monitoring (LaGier et al. 2007) and fast detection of biological warfare agents (Ivnitski et al. 2003). A variety of detection technologies have been utilized for DNA biosensors including optical (Gerion et al. 2003) and electrochemical (Cai et al. 2003) techniques. High sensitivity, selectivity, rapid analysis, ability to operate in turbid solutions and the possibility of miniaturization enabled electrochemical biosensors to become the

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most commonly used biosensors over other technologies (Shah and Wilkins 2003). Hence, in the literature numerous papers can be found that describe the electrochemical detection of DNA, proteins and microorganisms with very low detection limits (Barthelmebs et al. 2011; Bonel et al. 2011; Chen et al. 2010; Civit et al. 2012; Guo et al. 2013; Wang et al. 2008). However, it is notable that these data were generated using "home-built" electrochemical detection systems and assay conditions are mostly in static with very few assays performed with fluidic systems. For example, Skladal and colleagues immobilized antibodies on to the screen printed electrodes using conventional methods (static, overnight incubation). After the binding of the analyte and the enzyme bound secondary antibody, they placed the electrode to a flow chamber and subsequently substrate was injected to obtain an amperometric measurement during flow (Pohanka and Skladal 2007; Skládal et al. 2013). In another study, Jang and colleagues used electrode integrated poly(dimethylsiloxane) (PDMS) biochip for an immunoassay (Jang et al. 2006). Instead of using the electrode surface, they functionalized the PDMS channel surface with silane chemistry. All the assay steps including the immobilization and binding were performed during fluid flow and hence the assay procedure was reasonably fast with respect to the conventional methods. After the analyte binding, cyclic voltammetry technique has been applied to measure the substrate and enzyme reaction. In another study, Ben-Yoav and colleagues have performed the electrochemical DNA detection assay using a microfluidic chip and obtained nanomolar detection limit (Ben-Yoav et al. 2015). In another study Abad et al. has utilised microfluidics integrated microelectrodes for the detection of troponin T. In this case the assay has been performed in an eppendorf using magnetic beads and later the TMB reagent added assay mixture has been injected on to the electrodes for amperometric measurements (Abad et al. 2012).

In this work, we present a microfluidics and nanoparticles based amperometric biosensor for the detection of cyanobacteria nucleic acids. Some of the cyanobacteria produce protease inhibitor oligopeptides such as cyanopeptolins. For monitoring of the fresh water resources, a fast and sensitive biosensor assay has been described where cyanopeptolin coding region of the *Planktothrix agarhii* (NIVA-CYA 116) genome has been used as target. Unlike many DNA detection assays, all the steps of the assay including probe capture, hybridization and measurement have been achieved during reagent flow. The detection has been achieved using a biochip that has two sets of Au electrode arrays, each consist of shared reference/counter electrodes and 3 working electrodes (d = 1 mm). The components of the electrode array is large enough to be fabricated by means of a fine metal mask instead of photolithography, hence it is faster and cheaper to produce. At the same time the total size of the biochip is compact enough to fabricate a micro channel (capacity ~7 μl) over the electrodes. Thus, this platform enabled all the steps of the cyanobacteria detection assay including the amperometric measurement to be performed during the fluid flow allowing fast and sensitive readings.

2. Experimental

Phosphate buffered saline (PBS, 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4) tablets, mercaptoethanol, mercaptoundecanoic acid (MUDA), ethanolamine, spectrophotometric grade ethanol, horse radish peroxidase (HRP), 3,3',5,5'-Tetramethylbenzidine (TMB) ready to use reagent, hydrochloric acid (HCl), Tris-HCl, sodium chloride (NaCl), ethylenediaminetetraacetic acid (EDTA), N-hydroxysuccinimide (NHS), and potassium ferricyanide were purchased from Sigma-Aldrich (Poole, UK). 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and NeutrAvidin (NA) was purchased from Thermo Scientific. Gold nanoparticles (40 nm) were obtained from BBI International (Cardiff, UK). Potassium chloride (KCl) and biotin was purchased from Fisher Scientific (Loughborough, UK). *P. agardhii* NIVA-CYA 116 provided by the NIVA Culture Collection of Algae. Oxygen free argon was purchased from Habas (İstanbul, Turkey). Ultrapure water (18 MΩ cm^{-1}) was obtained from a Milli-Q water system (Millipore Corp., Tokyo, Japan). The oligonucleotide sequences were obtained from TIB Molbiol (Berlin, Germany): Target 5'- AGA CGT TAA TAC ATT GAA CCT GTT TTC CGA CCC ATT C -3'; Surface Probe Biotin-CAA TAT TTG GCG T GA ATG GGT CGG AAA ACA; Detection probe 5'- GGT TCA ATG TAT TAA CGT CTA TGG AAA – Biotin; Forward primer 5'- GTCGTAGCAGTGG-GAGGAGATGC-3'; Reverse primer 5'-AGGCTTCTGTAGGGCCATA-GACG-3'.

2.1. Electrochemical analysis

The biochip has been designed and fabricated on silicon dioxide wafer that consists of 2 × 3 working electrodes (d = 1 mm) each set has a shared Au counter and quasi-reference electrodes. A sensor cassette has been designed and fabricated from poly(methyl methacrylate) (PMMA) and a double sided sticky tape that formed a microfluidic channel on the electrode array. The capacity of the flow cell on electrodes was ~7 μl. Later, a potentiostat, syringe pump, injection valve and sensor chip docking station has been set up for the detection assays (Fig. 1). The operating temperature of the assays was 25 °C, and the flow rate of the buffer was 50 μl/min for the assays.

Cyclic voltammetry and amperometric measurements were

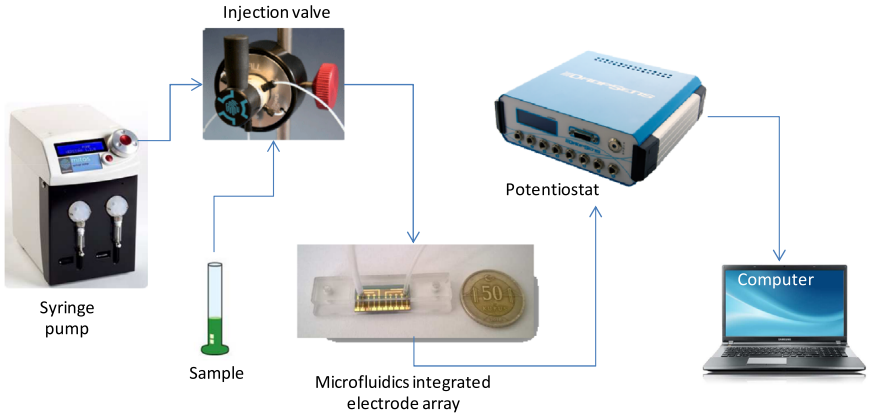

Fig. 1. Schematic illustration of the electrochemical detection system.

performed with a Dropsens MicroStat 8000 Electrochemical Analyzer with the general purpose electrochemical software Dropview 1.4 (Dropsens, Astuias, Spain). The electrochemical analyzer and the purpose built shielded cables enable simultaneous electrochemical measurements of 8 electrodes. Cyclic voltammetry (CV) tests were performed using 1 mM $K_4[Fe(CN)_6]$ solution in 1 M KCl. Amperometric detection was performed with a fixed potential of −0.1 V using TMB substrate system.

2.2. Preparation of HRP-NA labelled Au nanoparticles

In previous studies it has been shown that proteins can be simply adsorbed on the Au nanoparticle surface at their isoelectric point via electrostatic interactions (Uludag et al. 2010), (Ding et al. 2013). In the current study, the same procedure was implemented for the modification of the Au nanoparticles with PSA detection antibody and HRP. NA and HRP modified Au nanoparticles (40 nm) were synthesized by derivatizing 1 ml of aqueous Au nanoparticles with NA (1 or 1.5 μl) and HRP (1.5, 2.0 or 2.5 μl). The mixture was incubated for 45 min on a shaker at room temperature followed by centrifugation to remove excess reagents. The supernatant was removed; then 30 μl 10 mg/ml BSA and 100 μl Tris buffer were added. The modified Au nanoparticles were stored at 4 °C and warmed to room temperature before use. The concentration of nanoparticles was calculated by a spectrophotometer at 525 nm wavelength.

2.3. Surface modification

Surface modification of the electrode arrays with a self-assembled monolayer (SAM) has been achieved by plasma cleaning of the arrays and later immersing in ethanolic solution of 2 mM MUDA (80% or 40%) and 2 mM mercaptoethanol (20% or 60%) mixture for overnight (Navrátilová and Skládal 2004; Oh et al. 2004). Later the electrode arrays were rinsed with ethanol and water. After drying with nitrogen stream, the arrays were stored at +4 °C till use. SAM coated electrode array and the PMMA cassette were assembled together by means of a double sided sticky tape to form the sensor chip with a flow cell, and placed to the docking station to establish the electronic and fluidic connections. The sensor surfaces were prepared by immobilising NeutrAvidin on electrode array using conventional amine coupling chemistry. The running buffer used for immobilisation was degassed Dulbecco's modified phosphate buffered saline (PBS, pH 7.4) and this buffer continuously flowed over the sensor surfaces between the injections. The flow rate of the buffer/reagents for the assay was 50 μl/min unless written otherwise. Sensor surfaces were first activated with a 1:1 mixture of 400 mM EDC and 100 mM NHS, and mixed immediately prior to use (final concentrations; 200 mM EDC and 50 mM NHS). First 250 μl EDC-NHS, later 250 μl NA (50 μg/mL in PBS buffer) were injected across sensor surfaces. Non-reacted NHS esters were capped with 1 M ethanolamine, pH 8.5 (250 μl). After NA immobilisation, the running buffer was changed to Tris buffer comprising 20 mM Tris–HCl, 150 mM NaCl, 1 mM EDTA, pH 7.0. Biotinylated complementary surface probe (Table 1) was diluted in Tris buffer to 10 μg/ml and injected to the NA immobilised surface (250 μl) to obtain surface probe captured sensor surface. Finally, 10 mM Biotin in Tris buffer was injected for 1 min to block the remaining active sites of the NA layer.

2.4. Assay

The target sequence and detection probe were hybridized in a tube at 55 °C for 5 min at required concentrations; detection probe concentration being at least two fold higher concentration than the target sequence concentration. The resultant hybridized material (250 μl) was then injected over the sensor surface to be captured by surface probe (at 25 °C). Subsequently, NA and HRP modified Au nanoparticle solution (5×10^{10} nanoparticle/ml, 250 μl, 30 μl/min) were injected over the sensor surface. To obtain an electrochemical signal, a Real-time Electrochemical Profiling™ (REP) assay has been performed by applying −0.1 V potential to the electrode array and the current was measured continuously. Firstly current measurements were taken during Tris buffer injection and this signal was recorded as a baseline. The subsequent injection of 250 μl TMB incurred a current change and the subtraction between the current obtained during TMB and buffer was used as sensor response. Three data points were used to obtain the mean and standard deviation of the results. The limit of detection (LOD) was calculated as the signal obtained from the assay that is equivalent to the 3 times the standard deviation of the signals obtained from the blank standards.

2.5. PCR

After the nucleic acid extraction of the samples, the toxin producing region of the *P. agardhi* (NIVA-CYA 116) cyanobacteria (Neilan 2002) has been amplified (109 bp) by PCR using forward and reverse primers (Forward primer 5'- GTCGTAGCAGTGGGAGGAGATGC-3'; Reverse primer 5'-AGGCTTCTGTAGGGCCATAGACG-3'). 10 ng of genomic DNA was added to PCR mix (50 uM dNTPs, 1.5 mM $MgCl_2$, 10 pmol primers, 0.5 U Taq polymerase (Roche Diagnostics Co.)) and PCR was performed as follows: one cycle at 95 °C for 3 min; 30 cycles at 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s; and one final cycle at 72 °C for 5 min. The PCR products were analyzed on agarose gel in order to determine the PCR quality and cleaned with the Mini-Elute Kit (Qiagen Inc, Valencia, CA, USA). The amplified, PCR products were sequenced using Sanger sequencing system (Beckman Coulter) and quantified using a Qubit™ spectrophotometer with Qubit® dsDNA BR Assay Kit (Invitrogen). Prior to the on chip biosensor assay, PCR products have been denatured at 95 °C for 5 min, later primer 1 and detection probe have been added and allowed to hybridize at 55 °C for 10 min. Detection probe hybridized PCR products have been diluted to 1×10^{-9} M with Tris buffer (pH 7.0) and used as target DNA for the biosensor assay as described at the Section 2.4. PCR mixture containing enzyme, nucleotides and primers has been used as blank sample and treated with the detection probe as the target DNA has been treated prior to biosensor assay.

3. Results and discussion

3.1. Electrochemical characterization of sensor chip

To investigate the electrochemical behavior of the designed electrode arrays and to assess the coverage of SAM on electrodes, voltammograms were recorded for a model electroactive species, potassium ferrocyanide (1 mM in aqueous solution containing 1 M KCI; scan rate: $-0.1\ Vs^{-1}$). In order to have a compact and ordered SAM, the number of carbon atoms in the spacer structure need to be 10 or more (Bain et al. 1989). The molecular length of MUDA is longer than mercaptoethanol and when coated on an electrode surface, it creates an orderly SAM layer that can function as an isolating layer between the electrode and the electrolyte solution. However, mercaptoethanol is a shorter molecule with two carbon atoms, and hence allows electron transfer from the solution to the Au electrode surface. Therefore, by using MUDA and mercaptoethanol in different ratios, it is possible to obtain a SAM surface on electrodes where the ratio of electron transfer between the Au electrode and the liquid can be controlled. This allows to fabricate a sensor surface where electron transfer rate to the electrode and

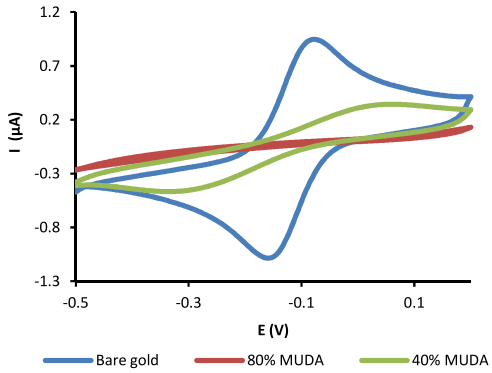

Fig. 2. Cyclicvoltammetry (CV) has been performed with 1 mM K4[Fe(CN)6]/KCl at 0.1 V/s scan rate using bare Au, 80% MUDA-20% mercaptoethanol coated, and 60% MUDA-40% mercaptoethanol coated Au electrodes.

the biomolecule immobilization capacity is optimised. In the study, two different SAM surfaces were created using 40% and 80% MUDA. While the electron transfer capacity of the SAM coated electrodes were investigated by cyclic voltammetry (Fig. 2), the assay performance of the electrodes were assessed by DNA detection assay (results shown at Section 3.4). The surface coverage (θ) of the self assembled monolayer can be calculated by comparing the charge (Q) during reduction/oxidation of cyclic voltammetry at a monolayer covered electrode with respect to a bare gold electrode (Eq. (1)) (Campuzano et al. 2006; Yang and Khoo 2004)

$$\theta = 1 - \left(Q_{SAM} / Q_{bare\ gold}\right) \quad (1)$$

Using Eq. (1), the surface coverage of 40% and 80% MUDA were calculated as 0.83 and 0.95 ± 0.03, respectively.

3.2. Au nanoparticle modification

In biosensors, such as immunosensors and DNA sensors, obtaining a high signal using very low concentrations of target molecule is a major goal, and use of nanoparticles provides a way to signal amplification to achieve this goal (Ding et al. 2013). HRP, antibody or NA modified Au nanoparticles have been used as one of the methods for enhanced immunoanalysis or DNA detection (Cui et al. 2008; Uludag et al. 2008). In previous studies it has been shown that proteins can be simply adsorbed on to the Au nanoparticle surface at their isoelectrical point via electrostatic interactions (Uludag et al. 2010). In the current study, the same procedure has been implemented for the modification of the Au nanoparticles with NA and HRP. The approximate number of proteins on a nanoparticle can be calculated with an assumed packing density of 0.907 (the circle packing density limit) (Schwartz and Quakea, 2007) using the Eq. (2) (the area covered by an antibody/protein = footprint area, calculated using the protein dimensions obtained from the PDB database)

$$\text{Number of antibodies} = 0.907 \times \frac{\text{nanoparticle surface area}}{\text{antibody footprint area}} \quad (2)$$

The approximate number of NA or HRP molecules that can fit on a 40 nm nanoparticle were calculated as 106 and 57, respectively, using the Eq. (2). In the study, the aim was to modify the Au nanoparticle with both proteins; NA was used for binding to the detection antibody, and HRP was used as a label for the electrochemical detection. In order to enhance the electrochemical signal further, the number of HRP molecules on a single nanoparticle need to be more than the NA molecules and hence the Au nanoparticle modification step needs to be optimized. For that purpose, HRP and NA at varying concentrations were used for Au nanoparticle modification and the performances of the nanoparticles were examined by adding TMB reagent and observing the color change due to the HRP content and by utilizing the nanoparticles for the DNA detection assay and observing the corresponding electrochemical signal. As seen from Fig. 3, all the NA-HRP modified Au nanoparticles showed color change after the addition of TMB indicating the successful conjugation of enzyme. The amperometric response obtained, after the binding of NA-HRP conjugated Au nanoparticles to the target/detection probe hybridized surface probe on the electrode. As seen from Fig. 3B, the conjugate prepared with 2 μl HRP and 1 μl NA (in 1 ml of Au nanoparticle solution), resulted in 50% and 21% higher amperometric current responses with respect to the other conjugates, and hence selected to be used for the further assays.

3.3. DNA detection

For a typical DNA detecting Real-time Electrochemical Profiling (REP™) (Olcer et al. 2014) assay, initially NA was immobilized on a self-assembled monolayer coated electrode array and subsequently biotinylated surface probe was captured, all by means of the fluid flow through the microfluidic system of the sensing platform. Later, the hybridized target and biotinylated detection probe were injected on to the sensor surfaces, following the capture of NA-HRP modified Au nanoparticles. At this point of the assay, the amperometric measurement was initiated to obtain the current measurements before and during the TMB reagent (enzyme substrate) flow (Fig. 4A and B). Initially, the DNA detection assay has been optimized with respect to the assay flow rate (800–400–200–100–30 μl/min), reagent volume and concentrations (neutravidin: 25–50–100 μg/ml, capture probe: 5–10–20 μg/ml). Optimal flow rate for the reagents were found as 30 μl / min for neutravidin immobilization, probe capture and target

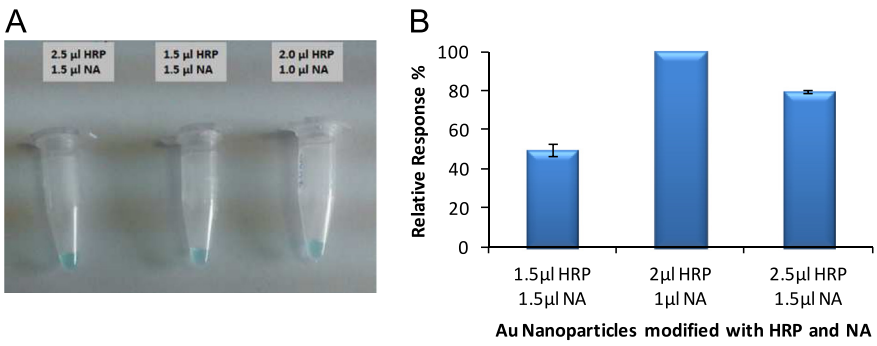

Fig. 3. (A) HRP and NA at varying concentrations were added to 1 ml Au nanoparticle solution for modification and the performances of the nanoparticles were examined by adding TMB reagent and observing the color change due to HRP content and (B) by utilizing the nanoparticles for the DNA detection assay and observing the corresponding amperometric signal.

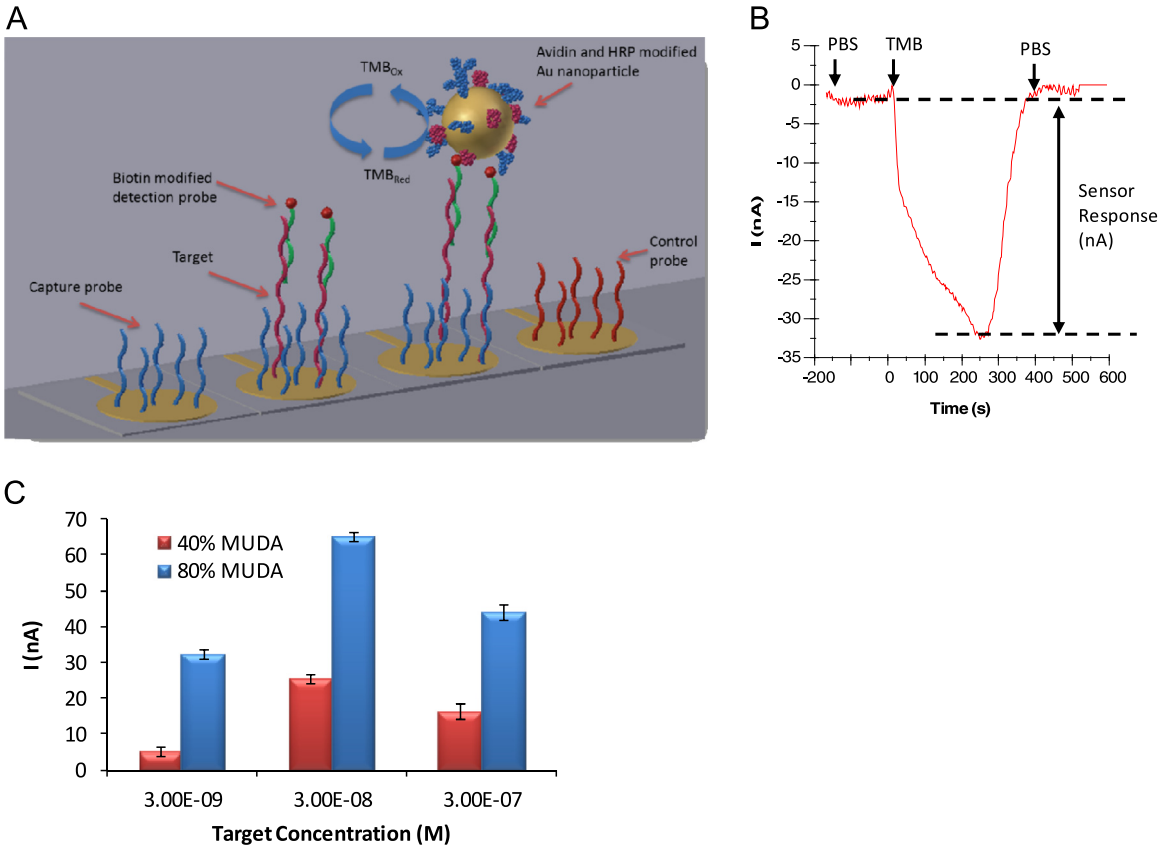

Fig. 4. (A) Schematic of the DNA hybridisation assay on electrode arrays. (B) The response vs time curve for the real-time amperometric measurement against −0.1 V potential. (C) REP response vs target DNA concentration plot for both 80% and 40% MUDA coated electrode arrays.

hybridization. For the regeneration of surfaces with 0.1 M HCl 100 μl / min has been found optimal, for the rest of the assay 50 μl / min was found optimal to get the required sensitivity from the sensors. Later, to obtain a calibration curve, target DNA at varying concentrations (6×10^{-12} to 3×10^{-7} M) were tested on the capture probe immobilised sensor surface. As control, without the target DNA, only NA-HRP modified Au nanoparticles solution and subsequently TMB reagent were injected over the probe captured electrode array during the amperometric measurement and 0.5 ± 0.4 nA non-specific binding was observed. The non-specific response was subtracted from the assay responses for target DNA detection. The DNA detection assay has been repeated using 40% and 80% MUDA coated electrode arrays, in order to investigate the best performing surface for the REP assay. By using MUDA and mercaptoethanol in different ratios, it is possible to obtain a SAM surface on electrodes with required conductivity and biomolecule immobilization capacity. With lower surface coverage (0.83), 40% MUDA coated electrode arrays may provide better conductivity that is good to conduct the signal generated by enzyme, whereas with a higher surface coverage (0.95), 80% MUDA coated electrodes may provide higher bio-molecule immobilization capacity, and hence may provide higher detection signals. To investigate which approach yields a better DNA detection, both surfaces were utilized for the DNA assay and found that, 80% MUDA coated sensors resulted in higher amperometric current response as seen for the Fig. 4C. This result suggest that the high surface coverage (0.95) of the 80% MUDA coated surface enables maximum bio-molecule immobilization and the remaining uncoated areas (0.05) are enough to convey the electrochemical response obtained from the surface bound enzyme / substrate pair to the Au electrode for the signal readout.

Later the cyanobacteria DNA detection assay has been performed using 80% MUDA coated electrode arrays to obtain a calibration curve for the target DNA concentrations 6×10^{-12} M to 3×10^{-8} M (Fig. 5A and B). The amperometric response of the assay has been calculated by two ways; the maximum current obtained 250 s after the substrate injection has been used as the sensor response or the response has been calculated as current per minute using the data between 50–100 s after the substrate injection (Fig. 5C and D). The obtained calibration curves yielded ~1000 times lower target DNA detection limit with respect to the 40% MUDA coated sensors with an LOD of 6×10^{-12} M and a linear correlation coefficient of 0.98–0.97.

In comparison with other electrochemical DNA biosensors, low detection limit was observed without long and complicated experimental procedure with REP assay (assay duration is 35 min (including 10 min in tube hybridisation of target and detection probes) after 28 min of PCR (including 5 min denaturation)). For example in another study, Meng et al. fabricated a novel biosensor based on enzymatic signal amplification via gold nanoparticles for DNA detection. After a long assay time (18 h) including immobilization and hibridization, they obtained 6.0×10^{-12} M detection limit (Meng et al. 2012). Wang et al. (2008) and Lin et al. developed electrochemical biosensor for detection of a fusion gene in acute promyelocytic leukemia with femto molar detection limit with 75 min assay time (Lin et al. 2011). In another study, an electrochemical genosensor was developed by Civit L et al. and used for the detection of human papillomavirus DNA sequences (Civit et al. 2010). Multiplexed detection of DNA sequences were achieved by using a designed array in nanomolar range. In another study, Laschi S et al. reported a new gravity-driven microfluidic platform using micromagnetic beads as a solid support which provided the detection of specific DNA sequences at nanomolar level and reduced analysis time to 16 min (Laschi et al. 2010).

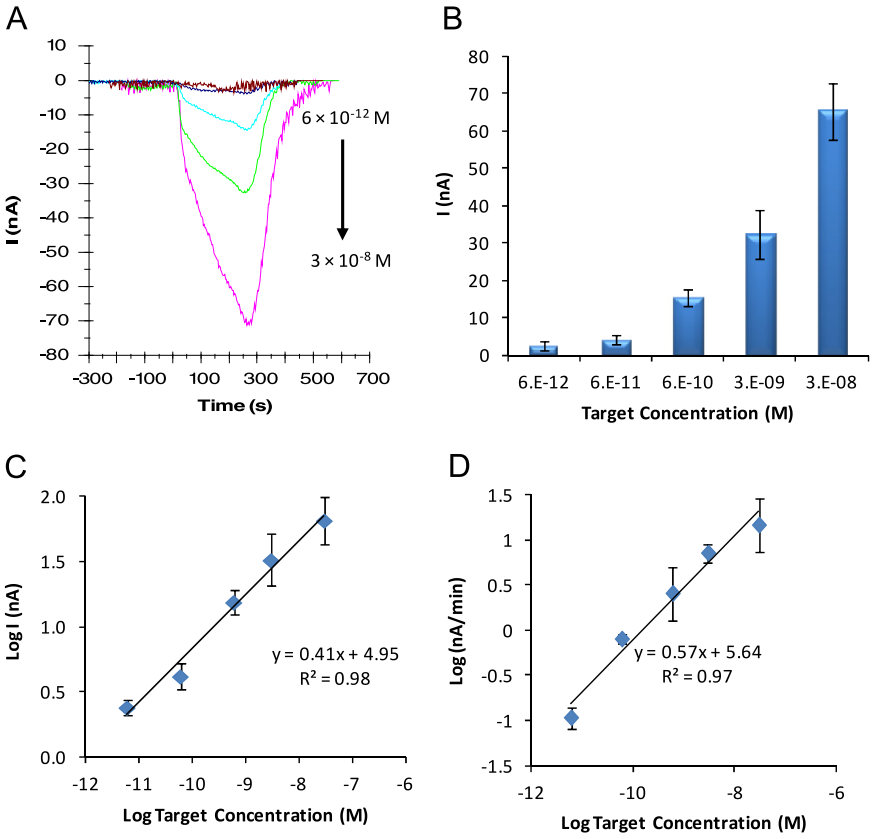

Fig. 5. (A) The plot shows the raw data of the REP responses of current vs time with respect to the target DNA concentrations (using 80% MUDA coated electrode arrays). (B)–(C) The calibration curves for the detection of target DNA (using 80% MUDA coated electrode arrays). The maximum current obtained 250 s after the substrate injection has been used as the sensor reponse. (D) The response has been calculated as current per minute using the data between 50–100 s after the substrate injection.

3.4. PCR product detection

The developed cyanobacteria detection assay has been utilized for the analysis of the PCR products. After the denaturation of the PCR products, both the biotinylated detection probe and the primer 1 have been added to the solution during the hybridization. This minimized the self-re-hybridization of the PCR products and hence allowed further hybridization of the target region of the PCR product on to the surface bound capture probe. Subsequent injections of the HRP and NeutrAvidin modified Au nanoparticles and the substrate resulted in an amperometric response. The developed assay enabled the detection of the *P. agarhii* PCR products indicating the potential of the biosensor for the detection of toxic microorganisms from the environmental samples (Fig. 6).

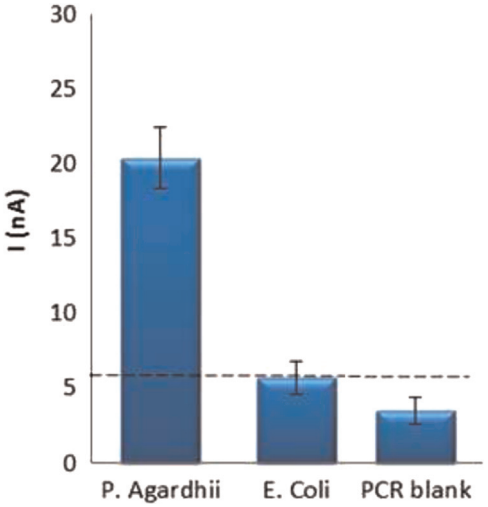

Fig. 6. The plot shows the REP responses obtained from the test of the PCR products (1×10^{-9} M): *P. agardhii* and negative control; *E. coli*.

4. Conclusions

Some of the cyanobacteria produce protease inhibitors such as cyanopeptolins, and cause drinking water contamination. Therefore, their detection has great importance to monitor the well-being of water sources that is used for human consumption. In the current study, to provide a rapid and sensitive detection of toxic bloom-forming cyanobacteria, a microfluidics and nanoparticles based amperometric biosensor has been utilized. The sensing platform comprises an electrode array; nanoparticle based sensing, detection in microfluidics and real-time amperometric measurements. All the steps of the assay including the immobilization of the capture DNA, hybridization of the target probe, capture of the enzyme bound Au nanoparticles and amperometric measurement have been performed during the fluid flow; hence there is no need for hours long hybridization/incubation periods and the sensing can be achieved reasonably fast. Using the developed assay, cyanobacteria nucleic acid detection has been achieved down to picomolar level within 25 min assay time. The developed assay not only enables the detection of the cyanobacteria with high-quality data, but also is suitable for automation and can be used to build a detection device for non-expert users for on-site testing. Therefore, further studies involve the design and fabrication of an automated on-site testing device for the detection of toxic microorganisms from the environmental samples.

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