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

Zehra Ölcer [a,b], Elif Esen [a], Aylin Ersoy [a], Sinan Budak [a], Dilek Sever Kaya [d], Mehmet Yağmur Gök [a], Serkan Barut [a], Duran Üstek [c], Yildiz Uludag [a,*]

[a] Bioelectronic Devices and Systems Group, UEKAE-BILGEM-The Scientific and Technological Research Council of Turkey (TUBITAK), 41470 Gebze, Kocaeli, Turkey
[b] Department of Chemistry, Gebze Technical University, 41400, Gebze, Kocaeli, Turkey
[c] Department of Medical Genetics & REMER, School of Medicine, Medipol University, Beykoz 34810 Istanbul, Turkey
[d] Fikret Biyal Research Center, Cerrapasa Medical Faculty, Istanbul University, 34098 Istanbul, Turkey

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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.

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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

* Corresponding author.
E-mail address: yildiz.uludag@tubitak.gov.tr (Y. Uludag).

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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'- GTCGTAGCAGTGGGAGGAGATGC-3'; Reverse primer 5'-AGGCTTCTGTAGGGCCATAGACG-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

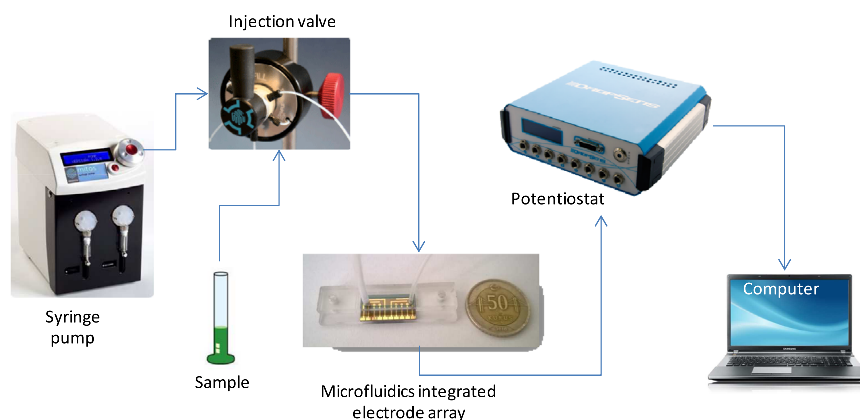

Fig. 1. Schematic illustration of the electrochemical detection system.

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