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Detection of bisphenol A using palm-size NanoAptamer analyzer

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ABSTRACT

We have demonstrated a palm-size NanoAptamer analyzer capable of detecting bisphenol A (BPA) at environmentally relevant concentrations (< 1 ng/mL or ppb). It is designed for performing reaction and fluorescence measurement on single cuvette sample. Modified NanoGene assay was used as the sensing mechanism where signaling DNA and QD_{655} was tethered to QD_{565} and magnetic bead via the aptamer. Aptamer affinity with BPA resulted in the release of the signaling DNA and QD_{655} from the complex and hence corresponding decrease in QD_{655} fluorescence measurement signal. Baseline characterization was first performed with empty cuvettes, quantum dots and magnetic beads under near-ideal conditions to establish essential functionality of the NanoAptamer analyzer. Duration of incubation time, number of rinse cycles, and necessity of cuvette vibration were also investigated. In order to demonstrate the capability of the NanoAptamer analyzer to detect BPA, samples with BPA concentrations ranging from 0.0005 to 1.0 ng/mL (ppb) were used. The performance of the NanoAptamer analyzer was further examined by using laboratory protocol and commercial spectrofluorometer as reference. Correlation between NanoAptamer analyzer and laboratory protocol as well as commercial spectrofluorometer was evaluated via correlation plots and correlation coefficients.

1. Introduction

Bisphenol A (BPA) is the epitome of irony from the Industrial Revolution. It was first synthesized and reported between 1890s and early 1900s (Einhorn, 1898). By the 1940s, it has become a hallmark of human ingenuity and innovation. It has allowed us to manufacture countless polycarbonate and epoxy resins based contraptions with excellent mechanical, thermal and chemical properties. These products ranged from inner lining of canned foods, thermal paper for receipts, plastic housings for electronics to toys and baby bottles. Yet in the next 70 years, the environmental and public health implications of BPA became increasingly apparent (Biedermann-Brem et al., 2008; Brede et al., 2003; Dodds and Lawson, 1936; Hoepner et al., 2016; Howe and Borodinsky, 1998; Peretz et al., 2014; Rykowska and Wasiak, 2006). Finally in the last 10 years, the regulatory authorities could no longer ignore or deny the jarring health risks posed by BPA and it has since been gradually phased out by manufacturers (Tullo, 2016). Unfortunately, the damage has already been done.

First reported by Dodds and Lawson (1936), BPA is now a well-known endocrine disrupting compound and it behaves like estrogen in biological systems and binds estrogen receptors. Since it mimics estrogenic hormones especially in endocrine systems, it can result in a wide host of serious health implications. They include reproductive and developmental disorders in infants and children, neurological disease, cancers, obesity, diabetes and cardiovascular diseases (Bodin et al., 2013; Lee et al., 2013; Maffini et al., 2006; Melzer et al., 2010; Nagel et al., 1999; Saal et al., 2012, 2005; Tharp et al., 2012; Yang et al., 2009).

Despite phasing out BPA in subsequent manufacturing processes, leachate from landfills as well as effluent from wastewater treatment plants continue to contaminate surface water bodies such as rivers and reservoirs with BPA (Flinta et al., 2012; Jackson and Sutton, 2008; Oehlmann et al., 2009). The estimated half-life of BPA in the effluent was 2.5–4 days (Dorn et al., 1987). Unfortunately with continuous leaching into the environment, BPA behaves like a pseudo-persistent contaminant. Note that it only takes a very low BPA concentration of 10^{-10}–10^{-8} mol/L or (0.02–2.28 ng/mL or ppb) to result in the above mentioned health implications (Salian et al., 2011; Sheng and Zhu, 2011; Vanderberg et al., 2009). Hence the perpetual contamination of water supplies by BPA has become one of the primary concerns of environmental and public health agencies.

In order to address above mentioned BPA contamination crisis, the

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availability of both laboratory based and portable BPA detection and quantification analysis has become invaluable. Established laboratory based analysis are currently well represented by gas chromatography with mass spectrometry (GC-MS), high pressure liquid chromatography (HPLC) (Ballesteros-Gómez et al., 2009; Nakazawa et al., 2014) and more recently by novel techniques such as that via DNA Y junction sensing method (Chen and Zhou, 2016). Immunoassay based analysis are attractive because they can be coupled with a variety of quantification techniques such as potentiometry, electrochemical measurement and evanescent wave excited fluorescence. However they were in turn highly dependent on the stability of antibody and susceptible to nonspecific binding with analog compounds as well as interference from ionic species (Marchesini et al., 2005; Ohkuma et al., 2002; Piao et al., 2007; Zhou et al., 2014). Conventional electrochemical detection lends itself very well to portability via screen-printed electrodes but remains vulnerable to interfering species (Alkasir et al., 2010). Ferric reagents based paper colorimetric BPA sensor is low cost and highly portable but is limited by its sensitivity and hence unsuitable for environmentally relevant concentrations (Park et al., 2016).

In the last 10 years, aptamer-based approach has been gaining popularity for the detection of BPA as well as other environmental toxins due to its specificity, sensitivity (Jo et al., 2011; Gao et al., 2016, 2017; Li et al., 2016a) as well as ability to couple itself to a variety of complimentary novel platforms such as Raman scattering nanoprobe (Marks et al., 2014), carbon nanofiber FET based sensor element (Kim et al., 2016), gold nanoparticles aggregation based colorimetry (Mei et al., 2013) as well as gold nanoparticles and quantum dots based fluorescence measurement (Li et al., 2016b). A noteworthy effort to develop a portable aptamer-based BPA detection system was undertaken by Yildirim et al. (2014) where a commercialized suitcase size evanescent wave based system (Long et al., 2009) modified for BPA detection.

In order to contribute to the above mentioned collective effort to address the massive issue of BPA contamination, we embarked on developing a palm-size NanoAptamer analyzer capable of BPA detection at environmentally relevant concentrations (< 1 ng/mL or ppb). As the basis for the presented work, we modified the NanoGene assay (Kim and Son, 2010; Kim et al., 2011) for BPA detection by replacing the probe DNA with a BPA specific aptamer and hybridizing it with the signaling DNAs. As shown in Fig. 1a, the QD_{565} encapsulated magnetic beads (MB) are conjugated by the formation of covalent bond with BPA specific aptamer. It is in turn hybridized with the signaling DNA – QD_{655} conjugate. Upon exposure to BPA (*incubation*), the signaling DNA – QD_{655} conjugate is released from the aptamer and removed (via *rinsing*). In this way, the decreased fluorescence signal from QD_{655} (*fluorescence measurement*) is indicative of the BPA concentration. In other words, as the BPA concentration increases, the corresponding measured fluorescence will decrease.

Specifically, we will first present the design and operation of the NanoAptamer analyzer with reference to its provision for on-system incubation, rinsing and fluorescence measurement. This is followed by baseline characterization with empty cuvettes, quantum dots and magnetic beads. Operation conditions in terms of rinse cycles as well as incubation duration as well as the necessity for cuvette vibration were also investigated. Using laboratory protocol as reference (off-system incubation, rinsing and fluorescence measurement by commercial spectrofluorometer), the capability of the NanoAptamer analyzer to detect and quantify BPA was sequentially demonstrated. This investigation culminated with on-system incubation, rinsing and fluorescence measurement by the NanoAptamer analyzer and its correlation with the laboratory protocol.

2. Materials and methods

2.1. Design and operation of NanoAptamer analyzer

The NanoAptamer analyzer is designed for single cuvette reaction and analysis (Fig. 1b and S1). The primary components consist of (i) a cuvette holder, (ii) a pair of miniature peristaltic pumps (Dolomite Miniature Peristaltic Pump, 3 V DC, 0.12 W, 0.45 mL/min, Dolomite Centre Ltd, Royston, UK) for transferring reagents, (iii) an articulated magnet (Neodymium, D42-N52, Disc 1/4″×1/8″, K & J Magnetics Inc, Pennsylvania, USA) mounted on a servo motor (TowerPro SG90 Servo, 4.8 V, Taiwan), (iv) a vibrating motor (Model Z7AL2B1690002, ~12,000 rpm, Jinlong Machinery and Electronics Co. Ltd, China) for reagent agitation, (v) LED array (10 W, 400–405 nm, Epiled, China) for quantum dots excitation, (vi) a pair of photodiodes for detection (S6430-01 and S6429-01, Hamamatsu Photonics K.K., Japan) and (vii) a charge integrator to measure the output from the photodiodes. The LED array is further fitted with a heat sink and fan to prevent overheating.

The charge integrator is custom-designed and fabricated. It employs a low cost precision operational amplifier (LTC1051, Linear Technologies, USA), 10 μF feedback capacitor and 2 kΩ feedback resistor (Figs. S2 and S3). Light is converted to electrical charges (electrons) by the photodiode and these electrical charges are accumulated in the feedback capacitor. The voltage drop across the feedback capacitor (hence that of the charge integrator output) is representative of the amount of light detected by the photodiode.

The components are controlled by an Arduino compatible Mega2560 microcontroller board via a 16×2 LCD and key pad (Fig. 2a). The LED array is powered via a parallel 9 V line with a series potentiometer. The potentiometer determines the final voltage of the LED array. The 9 V line (via a 5 V voltage regulator, LM7805A, Fairchild Semiconductor, USA) also powers both the fan and charge integrator. Electrical relays (TQ2-5V, Matsushita Electric Works, Japan) are used for switching the LED array as well as resetting the charge integrator. As shown in Fig. 2b, the NanoAptamer analyzer fits on a human palm.

During operation, the user controls the peristaltic pumps, servo motor and articulated magnet, vibrating motor and the LCD array via the key pad (Fig. S4). As mentioned earlier, the operation of the NanoAptamer analyzer can be divided in three key phases: (i) Incubation (ii) Rinsing and (iii) Fluorescence Measurement. The vibrating motor is used to ensure homogeneity during incubation as well as fluorescence measurement. This is further facilitated by suspending the cuvette holder from a plastic pivot. During fluorescence measurement, a cap is placed over the cuvette to minimize background light. The output from the charge integrator is from 0 to 5 V. In other words, it can be potentially read by the microcontroller board analog pins and display on the LCD as a reading. However in order to facilitate the ease of data collection in this study, a separate data logger (PCS10, 4-Channel Recorder, Velleman, UK) is used to record the output voltages from the charge integrator instead. The voltage output of the 540 and 660 nm photodiodes (via charge integrator) were recorded by data recorder as CH1 and CH2 respectively. During measurement, the normalized output signal of the NanoAptamer analyzer was calculated as follows:

$$\text{Normalizedoutputsignal} = \frac{\text{Voltage gradient of CH2}}{\text{Voltage gradient of CH1}}$$

The voltage gradient was obtained by dividing the change in output voltage from the charge integrator over the time duration.