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Inkjet-assisted layer-by-layer printing of quantum dot/enzyme microarrays for highly sensitive detection of organophosphorous pesticides

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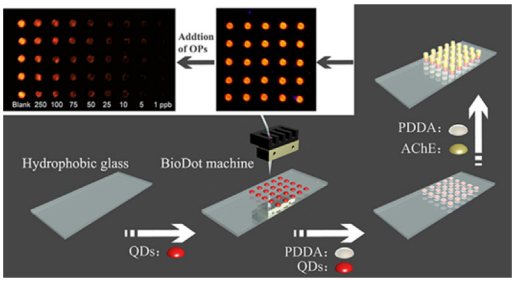
HIGHLIGHTS

- The large scale microarrays of CdTe QDs and AChE were fabricated by facile inkjet-assisted LbL printing technique.
- The QDs/AChE microscopic dot arrays could be used quantitatively and rapidly for the sensitively visual detection of OPs.
- A detection limit of 10 μg L^{-1} was achieved, much lower than levels specified by standard tests and other colorimetric detection methods.
- The low cost, short processing time, sufficient sensitivity, good stability and ease of use make it for a facile platform for on-site screening.

GRAPHICAL ABSTRACT



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ABSTRACT

We present a facile fabrication of layer-by-layer (LbL) microarrays of quantum dots (QDs) and acetylcholinesterase enzyme (AChE). The resulting arrays had several unique properties, such as low cost, high integration and excellent flexibility and time−saving. The presence of organophosphorous pesticides (OPs) can inhibit the AChE activity and thus changes the fluorescent intensity of QDs/AChE microscopic dot arrays. Therefore, the QDs/AChE microscopic dot arrays were used for the sensitive visual detection of OPs. Linear calibration for parathion and paraoxon was obtained in the range of 5−100 μg L^{-1} under the optimized conditions with the limit of detection (LOD) of 10 μg L^{-1}. The arrays have been successfully used for detection of OPs in fruits and water real samples. The new array was validated by comparison with conventional high performance liquid chromatography-mass spectrometry (HPLC-MS).

1. Introduction

Organophosphorous pesticides (OPs) are largely used in agriculture and fish hatcheries in the past several decades. However,

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their uncontrolled use has caused serious health and environmental problems throughout the world. Due to their high toxicity and potential to cause neurological disorders in humans [1–3], the development of rapid and reliable quantification of OPs in the food and environment has become imperative for the protection of public health and ecological systems. Although there are many conventional methods available for the detection of OPs, such as gas chromatography (GC), high performance liquid chromatography (HPLC), gas/liquid chromatography–mass spectrometry (GC/LC–MS) and capillary electrophoresis [4,5], which have high accuracy and sensitivity, they are not always convenient to employ such established analytical methods due to their high cost, long analysis duration and complicated operation procedures. Therefore, the development of a rapid, low cost, portable detection method is necessary to facilitate routine analysis with more convenience. Fortunately, biosensors based on acetylcholinesterase (AChE) or butyryl cholinesterase inhibition offer remarkable advantages over chromatographic methods, mainly in terms of simplicity, rapidly, reliability, low detection limits, and cost-effectiveness [6–13]. Thus it has become a simplified and portable alternative for OPs detection. In this type of biosensors, the enzyme inhibition-based colorimetric [14,15] or fluorimetric [16] screening methods for OPs determination have attracted increasing attention because of its low cost, simplicity and practicality. Since the color of the assays can be observed directly with the naked eyes or analyzed through a digital camera or typical DNA microarray scanners, colorimetric or fluorimetric screening methods does not require expensive or sophisticated instrumentation, and can be applied to field analysis and point-of-care diagnosis [17–19]. There are a number of enzyme inhibition-based colorimetric [14,15,20] or fluorimetric [16,21,22] assays available, however, these assays suffer from some limitations such as low sensitivity, the need for control of reaction conditions (pH and ionic strength) and utilization specific excitation wavelength etc.

To overcome these issues, we used semiconductor nanoparticles known as quantum dots (QDs) in place of organic dyes because QDs have unique optical properties including broad absorption with narrow photoluminescence spectra, high quantum yield, low photobleaching, resistance to chemical degradation, and are not sensitive to pH [23,24]. In this study, we report on the fabrication of large scale microarrays of CdTe QDs and cationic polyelectrolyte (poly(dimethyldiallyl ammonium chloride), PDDA) via facile inkjet-assisted layer-by-layer assembly (LbL) printing technique without a rinsing step [25–27]. The LbL microarrays of QDs/PDDA with relatively uniform surface morphology and red fluorescence were fabricated on hydrophobic surfaces. By further overprinting AChE/PDDA bilayers, the QDs/PDDA microarrays were developed to a robust fluorimetric assay for detection of OP residues. The fluorimetric assay is based on the reaction between the holes of CdTe QDs and thiocholine (TCh) generated by the AChE-catalyzed hydrolysis of acetylthiocholine (ATCh), which resulted in the fluorescence quenching of QDs/PDDA LbL microarrays. In the absence of OPs, AChE catalyzes the hydrolysis of ATCh, yielding TCh, which then quench the fluorescence of QDs through dissociation of photogenerated electron–hole pairs in QDs [28,29]. However, OPs can inhibit the enzyme activity, prohibiting the production of TCh. By analyzing the difference in fluorescence signal in the absence or presence of OPs, we are able to determine the concentration of OPs rapidly and accurately.

2. Experimental section

2.1. Materials

$Cd(ClO_4)_2 \cdot 6H_2O$, Al_2Te_3 powders and mercaptopropionic acid (MPA) were purchased from Alfa Aesar. AChE (EC 3.1.1.7 from *E. electricus*, lyophilized, specific activity 435 U mg^{-1}), acetylthiocholine chloride (ATCh), poly(dimethyldiallyl ammonium chloride) (PDDA, MW 478.9) and trimethoxy(octadecyl)silane (MW 387.93) were purchased from Sigma–Aldrich. The standard samples of paraoxon and parathion (100 μg mL^{-1} in acetone) were purchased from Beijing (WeiyekechuangKeji CO., LTD, China). MPA-capped CdTe QDs with PL peaks at 602 nm were synthesized according to our previous report (Figure S1 of the Supporting Information) [30]. Other chemicals of analytical grade were obtained from commercial sources and used as received. All solutions were prepared with ultrapure water from a Milli-Q water purification system (Billerica, MA).

2.2. Methods

The UV–vis absorption spectra were measured on a U-4100 UV–vis–NIR spectrometer (Hitachi, Japan), while the fluorescence spectra were recorded with a Fluoromax-4 fluorescence spectrophotometer (Horiba JobinYvon, Japan). An AD1510 (BioDot, America) printer with 15 μL printing cartridges was used for inkjet printing of all materials. A digital camera EOS 550D (Canon, Japan) was used to record images of the sensing arrays.

2.3. Fabrication of inkjet-assisted LbL microarrays of QDs/AChE

Inkjet-assisted LbL printing was conducted on different hydrophilic and hydrophobic substrates including glass, filter paper and polydimethylsiloxane (PDMS). The glass substrates was treated in hydrophilic or hydrophobic nature according to the following procedures: (1) The hydrophilic glass can be obtained by cleaning with Piranha solution (3:1 concentrated sulfuric acid and hydrogen peroxide mixture) according to the normal procedure used in our laboratory (Caution! Piranha solution is highly corrosive and toxic) [31]. (2) The hydrophobic glass was then obtained by functionalizing the cleaned glass with octadecyltrichlorosilane to yield an octadecyl-functionalized surface. Filter paper was treated with organic solvents to remove the intrinsic fluorescent species. For the preparation of hydrophobic substrates, a 90 wt.% PDMS solution was prepared by dissolving the silicone elastomer in toluene and shaking for 12 h and spun cast onto the silicon substrate. The coated films were subsequently cured in a vacuum oven at 80 °C for 12 h and carefully released from the silicon substrate.

As illustrated in Figure S2, the inkjet printing of the QDs/PDDA multilayer arrays was performed by alternatively printing 1 mg mL^{-1} positively charged PDDA solutions (pH 7.5, 0.5 M NaCl) and negatively-charged CdTe QD solution at the same position until the desired bilayer number was obtained. Subsequently, 1 mg mL^{-1} PDDA (pH 8.0, without NaCl) and 0.5 mg mL^{-1} negatively-charged AChE in PBS solution (20 mM) were alternatively printed at the same position in order to form three bilayers of AChE/PDDA on top of the QDs/PDDA multilayer microarray.

2.4. OP determination

The multilayer with a configuration of $(QDs/PDDA)_3(AChE/PDDA)_3$ was used for the detection of OPs. The procedure for OPs determination was as follows: (1) Different concentrations of OPs was first overprinted on the top of $(QDs/PDDA)_3(AChE/PDDA)_3$ microarrays and incubated at 38 °C for 15 min; (2) 20 mM ATCh was dropped onto the microarrays, and the fluorescence signal was then recorded after 5 min. The inhibition of OPs was calculated as following Eq. (1):