



Microfluidic origami nano-aptasensor for peanut allergen Ara h1 detection

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ABSTRACT

In this study, an origami microfluidic electrochemical nano-aptasensor was developed for the rapid detection of the peanut allergen Ara h1. Specifically, the microfluidic aptasensor was fabricated through sequential folding of a piece of chromatography paper substrate patterned with microchannel and screen-printed electrodes. Aptamer-decorated black phosphorus nanosheets (BPNs) were electrodeposited onto the paper-based electrode surface as sensing probes for enhanced electrochemical detection and high specificity and selectivity. Critical design parameters (the concentration of probe, time for self-assembly of aptamer and reaction time) were investigated to optimize the aptasensor performance. The prepared aptasensor was able to complete detection within 20 min and demonstrated a linear range from 50 ~ 1000 ng/mL with a detection limit of 21.6 ng/mL. The aptasensor was successfully used to detect the Ara h1 spiked cookie dough sample. The proposed method reduces the gap between complex lab testing and food allergen analysis at the point of need.

1. Introduction

Food allergy is an adverse immunologic response to a particular food (Iweala et al., 2018). The prevalence has increased in recent decades, although a complete understanding of the cause of the increase is still unknown (Sicherer & Sampson, 2018; Lopes & Sicherer, 2020). It is a severe and potentially life-threatening medical condition that influences the quality of life of allergic individuals (Rad et al., 2021). As one of the main causes of severe food-induced allergic reactions, peanut allergies bring huge psychological and economic burdens to patients (Du Toit et al., 2015). Ara h1 accounts for between 35 and 95% of allergic reactions in peanut allergy patients (Sobhan et al., 2018). Unfortunately, there is currently no cure for food allergies (Nance et al., 2020), and the only option for managing food allergies is strict avoidance of trigger foods (Jia and Evans, 2021). Exposures to undeclared allergens that can be inadvertently introduced into the food chain can pose a risk to food-allergic individuals (Remington et al., 2020). Therefore, it would be useful to have an accurate, rapid, low-cost food allergen detection device for use in the field or potentially by consumers.

Microfluidic paper-based analytical devices (μPADs) were first proposed in 2007 (Martinez, Phillips, Butte, & Whitesides, 2007). Compared with traditional materials in microfluidics such as crystalline silicon, glass and polydimethylsiloxane (PDMS), paper can achieve fluid

flow using its capillary force without any external equipment (de Castro et al., 2019; Li, Ballerini, & Shen, 2012). Recently, μPADs have been widely used in environmental monitoring (Lin et al., 2016; Liu, Hsu, Lu, Lin, & Ho, 2018), food safety (Busa et al., 2016; Zhang, Zuo, & Ye, 2015) and health diagnostics (Gong & Sinton, 2017; Li & Steckl, 2018) due to their advantages of portability, simplicity, economy and low sample consumption. Current quantitative analytical techniques used in μPADs include colourimetry (Shibata, Hiruta, & Citterio, 2019; Zhu et al., 2014), fluorescence (Scida, Li, Ellington, & Crooks, 2013; Zhang et al., 2019), chemiluminescence (Li et al., 2019; Wang et al., 2013), electrochemical detection (Cincotto, Fava, Moraes, Fatibello-Filho, & Faria, 2019; Cinti, Minotti, Moscone, Palleschi, & Arduini, 2017), electrochemiluminescence (Gross, Durant, Hipp, & Lai, 2017; Sun et al., 2018), and so on. Among these analytical methods, electrochemical detection technology has attracted significant attention because of its quantitative nature, high accuracy and sensitivity (Prasad et al., 2020; Wang et al., 2019). Combined with electrochemical biosensing, μPADs may be used to detect food allergens and significantly decrease the consumption of reagents and samples, improve the analysis speed, reduce the cost of testing and realize portability.

Traditional biosensors often use antibodies as recognition molecules, but the instability and high cost of antibodies limit the application of such biosensors (Seo & Gu, 2017). Compared to antibodies, aptamers

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obtained by SELEX (systematic evolution of ligands by exponential enrichment) are characterized by low cost, rapid analysis speed, high sensitivity and strong specificity (Kim, Raston, & Gu, 2016; Sampson, 2003). Moreover, aptamers are more easily modified and labelled by chemical methods, which is conducive to the functionalization of nanoparticles and their surfaces (Hassani et al., 2018). In recent years, the incorporation of nanotechnology into electrochemical biosensors has significantly improved detection performance (Manbohi & Ahmadi, 2019; Waheed, Mansha, & Ullah, 2018). BPNSs are a kind of graphene semiconductor material. Different from other semiconductor materials, BPNSs possess a direct and layer-dependent bandgap; thus, the thickness of BPNSs can be controlled to obtain specific bandgaps for various applications. In addition, BPNSs have high carrier mobility and considerable catalytic activity; therefore, they can be used as catalytic materials (Cho et al., 2016; Ge, Xia, & Guo, 2019).

In this paper, a 3D microfluidic origami nano-aptasensor was developed by integrating aptamers as recognition molecules and BPNSs to achieve simple, fast and accurate electrochemical detection of the peanut allergen Ara h1. A microfluidic origami chip in a two-dimensional flat geometry was first fabricated. An array of paper microchannels was formed on a paper substrate by PDMS patterning, while electrodes (working electrode/WE, counter electrode/CE and reference electrode/RE) were patterned on the substrate by screen printing of conductive inks. The 3D paper-based electrochemical aptasensor features a separate layer of WE via an origami pattern. The design of two WE sites allows for dual detection on a single microfluidic origami chip at a time. An aptamer specific to Ara h1 without labelling was employed as the molecular recognition probe. Meanwhile, a ferro-ferricyanide redox probe was used in the sensing process to achieve label-free electrochemical detection. Aptamer-functionalized BPNSs were then immobilized on the WE as probes. The specific binding between the aptamer and targeted allergen may cause a variation in charge transfer on electrodes and thus changes in current output. BPNSs were used due to their unique hinge structure, high bandgap and reactive edge structure (Ahmed et al., 2017) to enhance electrochemical signals and thus the sensitivity. The key operation parameters, namely, the concentration of aptamer deposited on the surface of WE, the time for self-assembly and aptamer-antigen reaction, were investigated to optimize the sensing performance of the aptasensor. To validate the performance of the proposed method, the major peanut allergen Ara h1 was selected as a testing model. The results demonstrate that improved sensitivity and selectivity are achieved compared to many reported methods, while no tedious or complicated procedures are required profiting from this paper-based microfluidic technique (Gezer, Liu, & Kokini, 2016; Peeters et al., 2014; Pollet et al., 2011; Singh, Sharma, Baltus, & Suni, 2010; Weng & Neethirajan, 2016).

2. Materials and methods

2.1. Reagents and materials

An aptamer against Ara h1 was selected by referring to a previous study (Weng & Neethirajan, 2016) and synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China). The sequence of which is as follow:

5'-TCG CAC ATT CCG CTT CTA CCG GGG GGG TCG AGC GAG TGA GCG AAT CTG TGG GTG GGC CGT AAG TCC GTG TGT GCG AA-3'

Conductive carbon ink (ED581ss) was provided by Tian Scientific Co. Ltd. (Shanghai, China). Ag/AgCl ink (CNC-01) was purchased from Jiangsu Linhe Transmission Technology Co., Ltd. (Xuzhou, China). A commercial screen-printed electrode (110) was purchased from Metrohm (Switzerland). Black phosphorus (BP), TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH = 8.0) and bovine serum albumin (BSA) were received from Dingsheng Technology Development Co., Ltd. (Chengdu, China). Ara h 2 and ovalbumin (OVA) were purchased from Sichuan Chenma Biotechnology Co., Ltd. (Chengdu, China). Magnesium chloride

hexahydrate ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$), phosphate-buffered saline (PBS) and other chemicals were all obtained from Solomon Biotechnology Co., Ltd. (Tianjin, China). Deionized water was used throughout the experiments. An average of four tests was conducted for each experiment, and the respective standard deviation is represented as an error bar to acquire statistical significance.

2.2. Preparation and characterization of an aptamer biosensing system based on BPNSs

The procured bulk BP (100 mg) was dispersed in 10 mL of deionized water, followed by vacuum degassing at 5 mBar to eliminate the dissolved oxygen molecules to avoid oxidation by lowering the pressure inside the tube containing the solution. Then, the mixture was sonicated using a bath sonicator (operating at 37 kHz and 150 W) in ice water for 24 h. Afterwards, the solution was centrifuged at 3500 rpm for 10 min. The supernatant was collected for further use. The morphological characteristics of BPNSs were observed by scanning electron microscopy (SEM, VEGA3, Hawke Guangyi International Co. Ltd, China). The SEM sample was prepared by drop-coating the well-sonicated BPNS dispersion onto a sample stub and air drying thoroughly.

The aptamer pellet was first resuspended in TE buffer to prepare a standard solution of 100 μM . Prior to use, aptamers must be folded into their active conformations for optimal binding. In this study, a series of dilutions of desired working concentrations were made from the standard solution in folding buffer (1 mM MgCl_2 , $1 \times$ PBS), followed by heating to 90 $^\circ\text{C}$ for 5 min and cooling to achieve folding. Afterwards, Ara h1 aptamers were conjugated onto polylysine (PLL)-functionalized BPNSs via electrostatic interactions. Here, PLL was used to turn the negatively charged surface of BPNSs positive to facilitate the immobilization of aptamers on the WE. BPNS supernatant with PLL (1 mg/mL, MW 30000–70000) was mixed at a ratio of 1:1 (v/v) thoroughly by shaking for 0.5 h followed by incubation in vacuum at room temperature overnight. An Ara h1 aptamer (5 μM) was added to an equal volume of BPNSs-PLL dispersion, mixed well and incubated at 4 $^\circ\text{C}$ for one hour to form the BPNSs-PLL-Apt bioconjugates. Characterization of the exfoliated BPNSs, BPNSs-PLL dispersion and BPNSs-PLL-Apt bioconjugates was performed by SEM and a Zeta and dynamic light scattering (DLS) system (90PlusPALS, Brookhaven, America).

2.3. Design and fabrication of microfluidic origami chip

A simple microfluidic origami chip for aptamer sensing for peanut allergen Ara h1 detection was designed and fabricated with chromatography paper (Whatman 1 CHR, Seebio®, Shanghai, China).

The microfluidic origami chip was fabricated through sequential folding of a piece of paper substrate with microchannels, pads for loading sample and patterning electrodes. The microchannel networks, including the sample delivery channel and those for patterning electrodes, were first drawn by Adobe Illustrator CC 2019 software (Adobe, San Jose, America), followed by printing on the surface of the chromatography paper substrate. A schematic diagram and picture of the proposed microfluidic origami chip are shown in Fig. 1A–Fig. 1C. In the design, a three-electrode system (WE, CE and RE) was used to perform the electrochemical measurement. The microfluidic origami chip consisted of two layers by folding (Fig. 1B) to avoid contamination during the functionalization procedure on the WE. As noticed in the figure, two independent detection sites were made to allow dual-target detection or screening at a time. To form a microfluidic origami chip, the WE parts were folded downwards and aligned to the middle of the top detection area. Tails were fabricated to connect the patterned electrodes to the electrochemical workstation via electrical conducting connectors. A small gap between WE and RE was designed to reduce the uncompensated resistance between them. A large area of CE was designed to allow unlimited current transmission. Liquid PDMS was coated and carefully swiped on the surface of the substrate except for the sample pad,

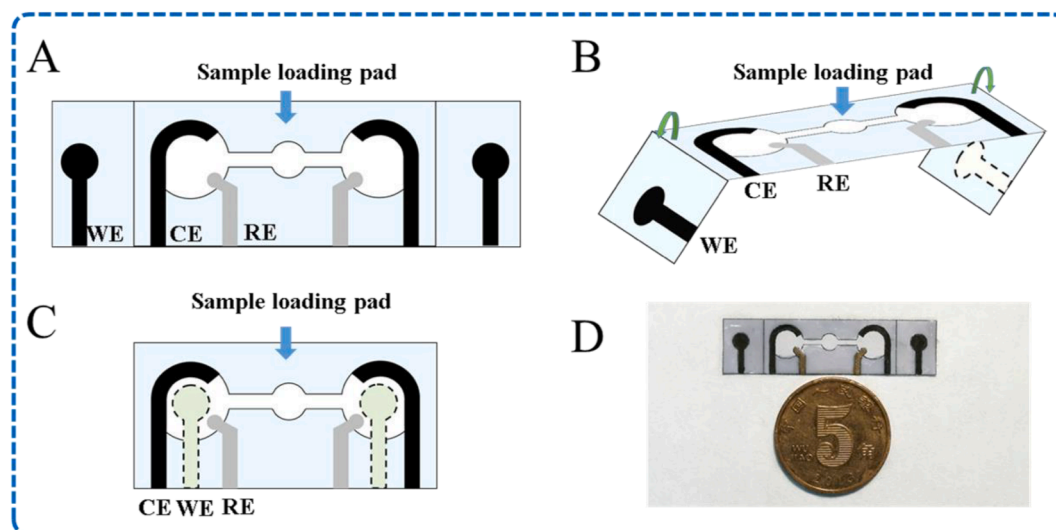


Fig. 1. Schematic (A ~ C) and picture (D) of the microfluidic origami electrochemical aptasensor.

delivery channels and electrode patterning area. The liquid PDMS would flow into the substrate in a few seconds due to the porosity. The substrate was then heated at 60 °C for 5 min to solidify the PDMS to form the hydrophobic wall. The tailed channels of the three electrodes were treated in the same way as PDMS to become hydrophobic to prevent direct contact between the solution and electrical connectors. A screening-printing method was used to fabricate the desired electrodes. The WE and CE were printed with conductive carbon ink, while RE was printed with conductive silver/silver chloride ink. After the electrodes dried out, a microfluidic origami chip was ready for use, as shown in Fig. 1D. The diameter and width of the WE and tail channel were 3 mm and 2 mm, respectively. The dimensions of the sample pad and liquid delivery channels were 3 mm in diameter and 1 mm and 10 mm in width and length, respectively.

2.4. Electrode modification

Working electrodes were modified by the following steps. First, 5 μ L of BPNSs-PLL suspension was dropped onto the surface of the WE and then left to dry in a vacuum at room temperature for 30 min. Subsequently, 5 μ L of aptamer solution was transferred onto the surface of the

BPNSs-modified WE and incubated in a vacuum at 4 °C for 1 h. Afterwards, 5 μ L of blocking buffer (BSA, 1%) was added to the modified WE surface and incubated at room temperature for 10 min to block the possible active sites on the WE surface to avoid nonspecific absorption. The WE surface was gently washed with PBS and deionized water successively. Modified aptasensor was stored at 4 °C for future use.

2.5. Electrochemical measurement of Ara h1

A schematic of the sensing mechanism of the proposed microfluidic origami chip is shown in Fig. 2. Electrochemical detection was performed via a three-electrode system. A ferro-ferricyanide redox probe was used in the system, hence making it a label-free electrochemical biosensor. The redox reaction of the probe occurred under an applied external voltage, resulting in an electrochemical signal. In this study, we used cyclic voltammetry (CV) for qualitative analysis and differential pulse voltammetry (DPV) for quantitative measurement. The specific binding of the Ara h1 antigen and aptamer formed a resultant complex, which caused diffusion hindrance on the WE and thus a corresponding change in the electrochemical signal. Ara h1 of higher concentration produced greater diffusion hindrance on the surface of WE and greater

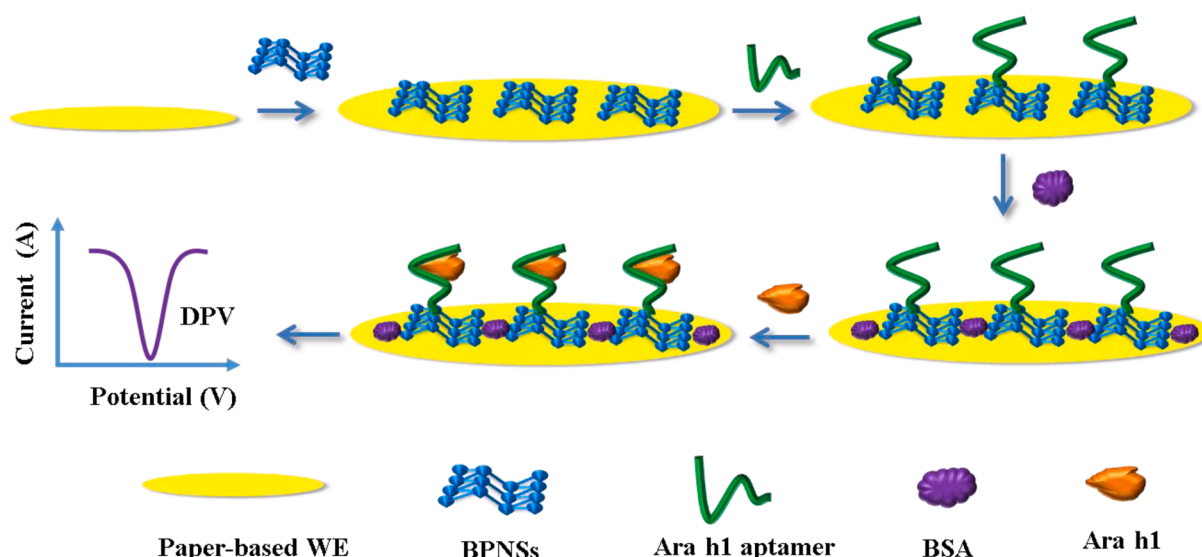


Fig. 2. A schematic illustration of the aptamer sensing mechanism of the microfluidic origami electrochemical aptasensor.

change in DPV peak current. The variation in peak current (ΔI) of the DPV signals before and after the binding reaction associated with the amount of the resultant complex was used to quantitatively determine the concentration of peanut allergen Ara h1.

Electrochemical measurements were performed by an electrochemical workstation (CHI660E, Shanghai Chenhua Instruments, China). Electrochemical characterizations of the modified surface were investigated by CV and DPV measurements carried out in a redox probe solution of 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl solution. CV scans run between -0.2 V and 0.6 V with a setting of 0.05 V s^{-1} scan rate. DPV scans were conducted between -0.1 V and 0.4 V with a step increasing potential of 0.004 V, modulation amplitude of 0.05 V, a pulse width of 0.06 s, sampling width of 0.02 s and pulse period of 0.5 s.

2.6. Preparation of spiked cookie dough sample

To validate the performance of the proposed aptasensor, the peanut allergen Ara h1 was selected as the detection target in this study. Peanut-free cookie dough samples were purchased from a local grocery store. Spiked cookie dough sample preparation was performed according to our previous study (Weng & Neethirajan, 2016). One gram of the homogenized cookie dough sample was ground and mixed with 20 mL PBS buffer, followed by incubation at 60°C for 15 min in a water bath. Then, the solution mixture was centrifuged at 4000 rpm for 5 min to collect the supernatant. After being filtered by a filter syringe of $0.2 \mu\text{m}$ diameter pores, the supernatant was diluted 1:10 with PBS buffer. The supernatant dilution was then spiked with Ara h1 allergen into a series of working solutions with theoretical concentrations between $200 \sim 800 \text{ ng/mL}$ for further measurement.

3. Results and discussion

3.1. Characterization of BPNSSs-PLL-Apt bioconjugates

Chromatography paper was selected to fabricate the microfluidic chip due to its superior porosity and hydrophilicity. The morphological characteristics of the exfoliated BPNSSs were explored by SEM imaging. As shown in Fig. 3A, the exfoliated BPNSSs had a typical sheet-like pattern and layered structure. The layered structure was formed by interlayer interactions and strong in-plane bonds between BPNSSs due to weak van der Waals forces (Stanford, Rack, & Jariwala, 2018). This stacking-up layered structure made it a stable 2D nanosheet material. PLL-modified BPNSSs and BPNSSs-PLL-Apt bioconjugates were characterized via a zeta potential and DLS system. The zeta potential and size distribution of the functionalized BPNSSs were measured. Fig. 3B shows that the surface potential of BPNSSs increased from -17.2 mV to $+5.6 \text{ mV}$ after modification of PLL due to the attachment of positively charged PLL molecules. After immobilization of aptamers, the zeta potential declined from $+5.6 \text{ mV}$ to -26.69 mV , indicating the successful binding of the negatively charged aptamers. The measured average diameters of the BPNSSs, BPNSSs-PLL and BPNSSs-PLL-Apt bioconjugates were 434.81 nm , 1050.21 nm and 7453.16 nm , respectively. All measurements mentioned above indicate the successful functionalization of the aptamer on the BPNSSs.

3.2. Validation of the aptasensing mechanism

The aptasensing mechanism was first validated using a commercial screen-printed electrode via CV measurement. $5 \text{ mM } [\text{Fe}(\text{CN})_6]^{3-/4-}$ was used as a redox probe. The concentration of the aptamer and time for self-assembly used to functionalize the WE were $5 \mu\text{M}$ and 30 min ,

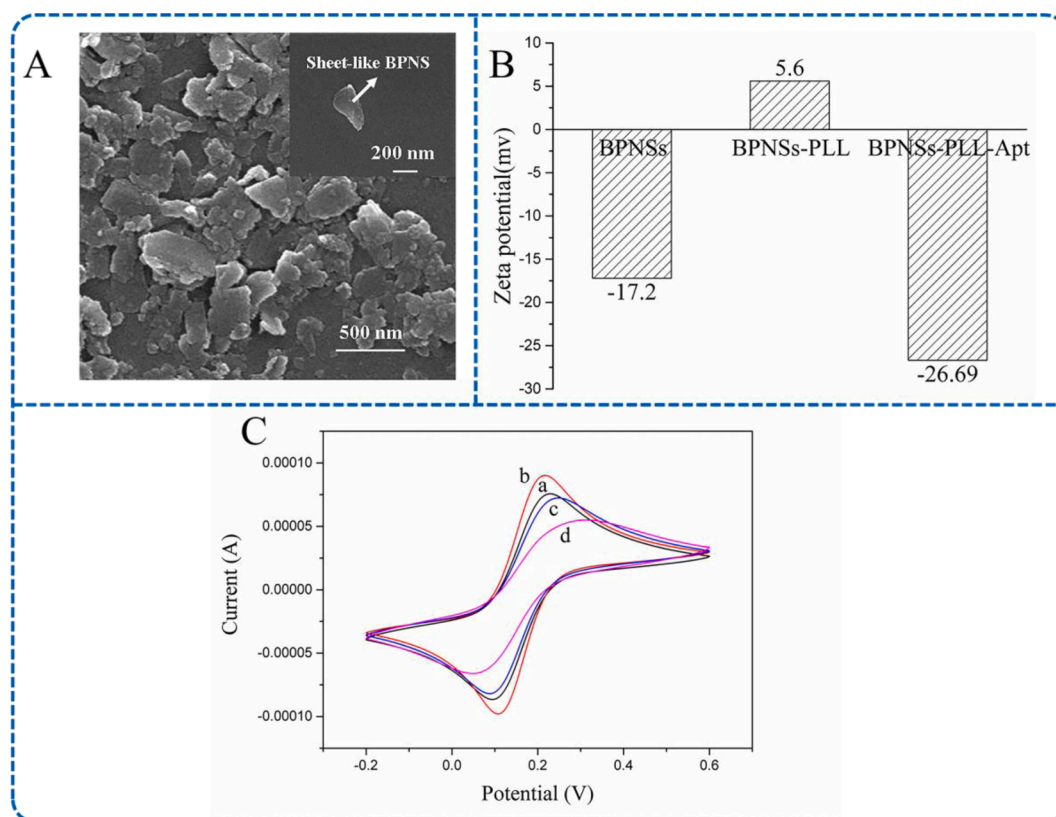


Fig. 3. Characterization of BPNSSs, BPNSSs-PLL and BPNSSs-PLL-Apt bioconjugates. (A) SEM image of the BPNSSs. (B) Surface zeta potential and (C) CV characterization of the aptasensor on a commercial screen-printed electrode. Curve a: CV response of the commercial electrode with bare WE. Curve b: CV response of the commercial electrode with BPNSS-modified WE. Curve c: CV response of the commercial electrode with BPNSSs-PLL-Apt-modified WE. Curve d: CV response of the commercial electrode upon the detection of Ara h1 at 800 ng/mL .

respectively. An Ara h1 aliquot of 800 ng/mL was detected, and CV measurement was performed with a reaction time of 20 min. The CV characterization results of the process are shown in Fig. 3C. Curve a is the CV response of the bare WE. After modifying the WE with BPNSs, the CV signal increased significantly (Curve b) because of the excellent conductivity of the BPNSs. Due to the steric hindrance of the aptamer on the WE surface, the CV signal decreased (Curve c) upon immobilization of the Ara h1 aptamer. After adding the Ara h1 antigen solution, the binding of the Ara h1 antigen and aptamer further increased the steric hindrance of the WE surface, resulting in the reduction of the electrochemical signal again (Curve d). The CV characterization results of the commercial screen-printed electrodes prove that the electrochemical aptasensing mechanism works well.

3.3. Optimization of the aptasensor parameters

To improve the performance of the proposed electrochemical aptasensor, several core parameters were optimized, including the concentration of aptamer immobilized on the surface of WE, time for self-assembly of aptamer and antigen-aptamer reaction time. DPV analysis was used due to its high sensitivity in quantitative analysis. For an accurate evaluation, the ratio of the relative changes of the DPV peak value on the aptasensor during the reaction and the initial peak current value ($\Delta I/I$) under the specific parameter settings were used. A greater value of $\Delta I/I$ indicates an adequate reaction between the aptamer and allergen on the WE, while a lower value indicates an inadequate reaction that may be caused by the insufficient coating of aptamer probes or immobilization/reaction times.

Various periods for the self-assembly of the aptamer in a range of 0.5 ~ 4 h were investigated to obtain a high efficiency of the self-assembled aptamer, while other parameters were kept the same throughout all the tests. The experimental results (Fig. 4A) show that an increase in the relative changes was observed along with the extension of self-assembly time. Although prolonging the self-assembly time of the aptamer could

increase the electrostatic interaction between the negatively charged aptamers and the BPNSs modified on the surface of WE, the DPV signal increased slightly and tended to be stable after 1 h, indicating the completion of electrostatic interaction on the binding sites of WE within a certain period. Therefore, 1 h was selected as the optimal time period for the self-assembly of the aptamer.

To improve the aptamer coverage on the surface of WE as well as the detection sensitivity of the aptasensor, various aptamer concentrations in a range of 1 ~ 10 μM were studied while other parameters were kept the same throughout all the tests. As shown in Fig. 4B, when the concentration of aptamer increased from 1 μM to 5 μM , the relative change in the DPV peak value increased significantly, demonstrating a decrease in DPV signal variation after the reaction due to diffusion hindrance caused by the Ara h1 antigen-aptamer complex on the WE. However, for concentrations above 5 μM , a decrease in the relative change in the DPV peak value was observed, indicating less binding reaction between the aptamer and antigen. The reason for this may be that the accumulation of high concentrations of aptamers occurs on the surface of WE and that the stacked aptamers affect subsequent binding events. Therefore, it can be concluded that 5 μM is the concentration required to generate current saturation for the proposed aptasensor. To avoid waste and high resistance, 5 μM aptamer was selected for further investigation.

The reaction time of antigen and aptamer can dramatically affect the binding degree and the accuracy of the electrochemical signal. Hence optimizing the interaction between antigen and aptamer on WE surface helps in achieving effective sensing. An incubation time of 5 ~ 30 min was evaluated. Fig. 4C shows that when the binding time reached 20 min, the decrease in current reached the maximum, and afterwards, no significant binding occurred in the case of a longer period. Therefore, 20 min was selected as the optimized time span for antigen-aptamer binding in the final experiment.

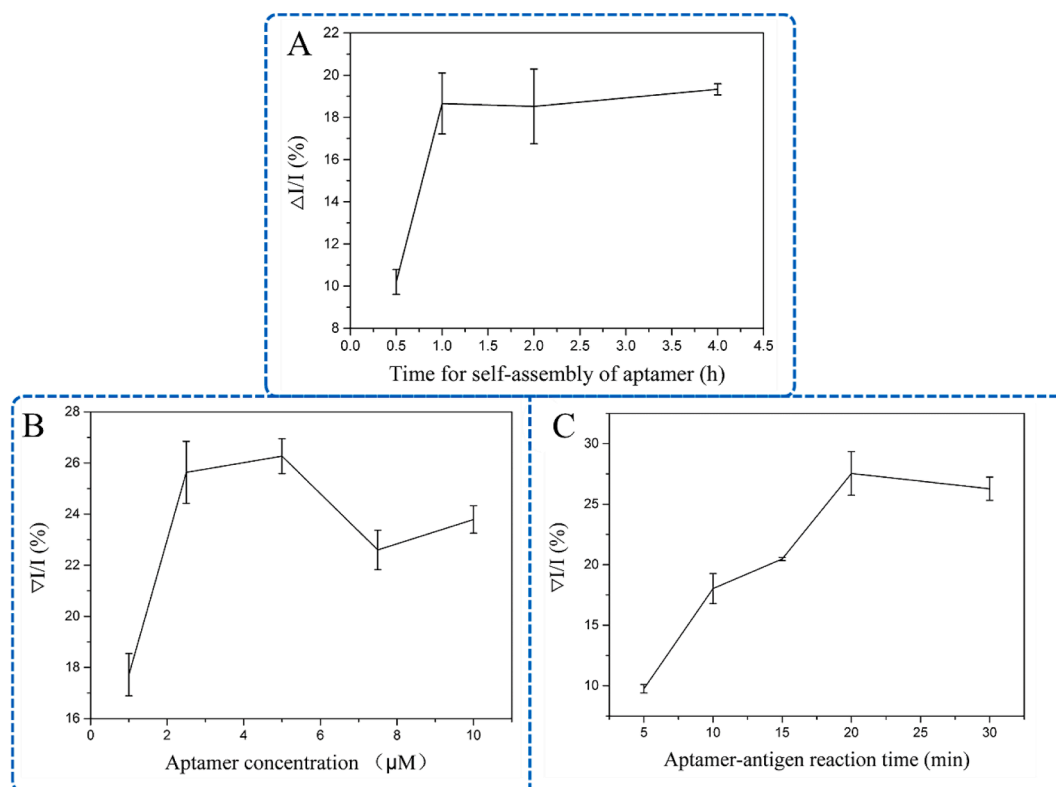


Fig. 4. Optimization of the paper-based electrochemical microfluidic aptasensor. The ratio of relative changes of the DPV peak current on the aptasensor obtained from (A) various time periods for self-assembly of aptamer (B) various concentrations of aptamer (C) various reaction time between aptamer and Ara h1 antigen.

3.4. Paper-based microfluidic Ara h1 aptasensing

Microfluidic devices have proven to be great platforms for biomedical applications with low sample/reagent consumption, faster detection and high sensitivity. In this work, a paper-based electrochemical microfluidic chip was developed to detect the food allergen Ara h1. The sensing mechanism of the microfluidic aptasensor was based on aptamer-targeted antigen binding (Fig. 2). The DPV signal was used to analyse the sensitivity and specificity of the proposed Ara h1 aptasensor. With the optimized parameters, DPV signals were measured for Ara h1 detection in a solution of 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ (0.1 M KCl solution). The binding of the aptamer and Ara h1 allergen may result in a reduction in the peak current, which is generated by the steric hindrance of the WE surface. The variation in peak current (ΔI) before and after the reaction as a function of the target concentration was used to determine the concentration of the sample. To obtain a calibration curve, a series of standard Ara h1 solutions ranging from 50 ng/mL to 1000 ng/mL were measured. For each data point, an average of four trials was performed to acquire statistical significance. As shown in Fig. 5, the peak current variation increased gradually with the increasing concentration of Ara h1 due to the conformational change of the aptamer on the WE in the presence of the target allergen. A good linear relationship was achieved between 50 ng/mL and 1000 ng/mL Ara h1 allergen with a correlation coefficient of $R^2 = 0.99507$. High sensitivity of 0.05 $\mu\text{A}\cdot\text{ng/mL}$ was obtained. The detection limit of the aptasensor was 21.6 ng/mL by $3s_b/S$, in which the standard deviation of the blank measure and sensitivity are represented by s_b and S , respectively (Thomsen et al., 2003). This detection limit is superior to that of many other biosensors for Ara h1 detection, for example, 56 ng/mL (Weng & Neethirajan, 2016) and 0.14 mg/mL (Gezer, Liu, & Kokini, 2016), as reported. In the experiments, each concentration was measured four times. All relative standard deviations were within 4.2%, indicating acceptable repeatability. To further verify the stability of our aptasensors, they were stored at 4 °C without any treatment. Three aptasensors were taken every other day to measure the electrochemical signals, and a period of one month was tested. With an increase in storage time, a variation of less than 2% was obtained, demonstrating the good stability of our aptasensors. All these results indicate the good sensitivity, accuracy, repeatability and stability of the proposed microfluidic aptasensor.

3.5. Specificity studies and spiked cookie dough sample analysis

Cross-reactivity testing is essential for the performance evaluation of a biosensor. To examine the specificity and selectivity of the proposed

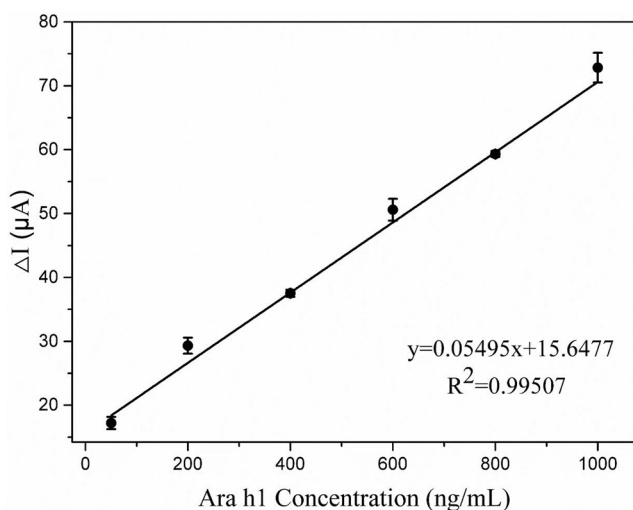


Fig. 5. Linear fitting of the peak current variation (ΔI) for various Ara h1 concentrations (50 ~ 1000 ng/mL) with $R^2 = 0.99507$.

microfluidic aptasensor towards Ara h1, some interfering proteins, including Ara h2, ovalbumin (OVA) and negative control (PBS buffer), were tested. Proteins of the same concentration level (400 ng/mL) prepared in PBS buffer and negative control were loaded onto a microfluidic aptasensor followed by incubation and DPV measurements. All tests were performed under the same analysis conditions, and four tests were performed for each data point to acquire statistical significance. As shown in Fig. 6, a huge peak current change was presented for Ara h1, while very few changes were observed for the other two proteins and the negative control. The result demonstrates that no interaction exists between the recognition aptamer probe and other proteins, thus presenting good specificity of the microfluidic aptasensor to detect peanut allergen Ara h1.

The accuracy of the aptasensor was further evaluated by detecting Ara h1 in spiked food samples. The recovery rate was calculated to evaluate the performance of the aptasensor. The cookie dough sample extracts were prepared by the procedure explained in Section 2.6. The extracts were then spiked with standard Ara h1 solution to form the theoretical known concentrations (200 ~ 800 ng/mL). The spiked working samples were tested by following the standard procedure as previously mentioned. An average of four trials was performed to acquire statistical significance. The experimental results are listed in Table 1. As presented, great consistency was obtained. The recoveries of the proposed aptasensor were in the range of 98.3–107.9% with relative standard deviations less than 5%, which proves the reliability of our microfluidic aptasensor in spiked food sample analysis.

4. Conclusions

In this study, we demonstrated a microfluidic electrochemical aptasensor that combines low-cost (USD \$0.8) rapid fabrication on paper and an aptamer-decorated BPNS probe to detect the peanut allergen Ara h1. Taking advantage of the good electroconductivity property and large surface area of BPNSs, the aptasensor demonstrated enhanced performance towards aptamer antigen-based immunosensing in a wide linear range of 50–1000 ng/mL with a high sensitivity of 0.05 $\mu\text{A}\cdot\text{ng/mL}$, a detection limit of 21.6 ng/mL, and high selectivity and specificity. An average time of 20 min was required for typical detection by the proposed aptasensor. It was also effective in determining peanut allergen Ara h1 in the spiked cookie dough sample with an acceptable recovery rate and relative standard deviation values ranging between 0.9% and 3.6%. Therefore, this simple, low-cost and easy-to-use microfluidic electrochemical nano-aptasensor offers a promising platform for detecting the peanut allergen Ara h1 in point-of-need tests.

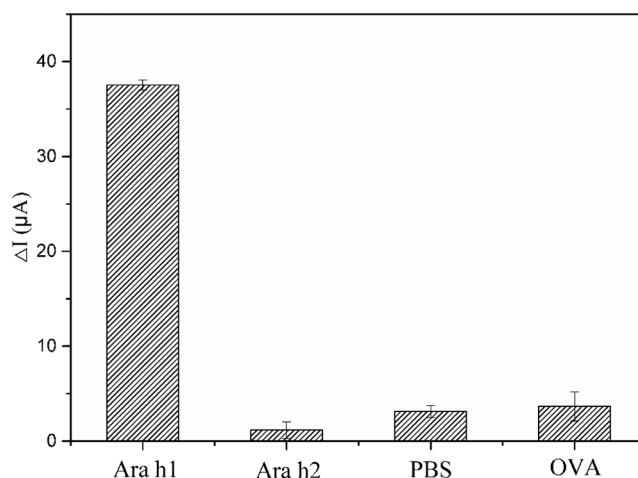


Fig. 6. Cross-reactivity testing of the microfluidic aptasensor against interferents (Ara h2, OVA and PBS).

Table 1

Determination of peanut allergen Ara h1 concentration in the spiked cookie dough sample by the proposed microfluidic origami aptasensor.

Spiked Concentration (ng/mL)	Measured Concentration (ng/mL)	Recovery Rate (%)	RSD (%)
200	215.7 ± 18.2	107.9	3.6
400	408.6 ± 10.9	102.2	1.6
600	629.7 ± 8.2	104.6	0.9
800	786.3 ± 31.9	98.3	3.0

CRediT authorship contribution statement

Hai Jiang: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Software, Supervision, Validation, Project administration, Writing - original draft, Writing - review & editing. **Qian Guo:** Data curation, Investigation, Methodology, Validation, Software, Writing - original draft, Writing - review & editing. **Cheng Zhang:** Investigation, Methodology, Software, Validation, Writing - review & editing. **Zhikang Sun:** Methodology, Visualization, Software, Writing - review & editing. **Xuan Weng:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Supervision, Project administration, Resources, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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