Silicon phthalocyanine-decorated TiO₂ mesocrystal coupled with multifunctional all-carbon structure for multistep cascade signal amplifier in photoelectrochemical immunoassay

Yilin Li,^a Shupeí Zhang,^a Hong Dai,^{*a} Yanyu Lin,^b Baoshan Zeng^a and Zhensheng Hong^c

a College of Chemistry and Chemical Engineering, Fujian Normal University, Fuzhou 350108, P. R. China, Fax: (+86)-591-22866135, E-mail: dhong@fjnu.edu.cn.

b Ministry of Education Key Laboratory of Analysis and Detection for Food Safety, and Department of Chemistry, Fuzhou University, Fuzhou 350002, P. R. China

c Shenzhen Key Laboratory of Transformation Optics and Spatial Modulation, Kuang-Chi Institute of Advanced Technology, Shenzhen, Guangdong 518057, China.

Abstract

Herein, a new photoelectrochemical sensor for aflatoxin B₁ (AFB₁) detection was established based on the all-carbon nanohybrids as transducer scaffold accompanied with quasi-octahedral TiO₂ mesocrystal (QOTM) absorbed dendritic Si phthalocyanines (SiPcs) as the efficient bioprobe. Specifically, the all-carbon nanocomposites exhibited fine photoelectric properties due to the photo-generated electrons of carbon quantum dots could be rapidly accepted by conductive carbon nanohorns, which evidently expedited the electron transport, and the nanohybrids were first employed as affinity support to anchor AFB₁ antibody. The dendritic SiPcs sensitized QOTM presented better photocurrent response and stability in comparison with QOTM alone due to impressive light-harvesting capability of the dendritic SiPcs. Under light irradiation, the photo-induced electrons of SiPcs migrated to the QOTM, and successively transferred to carbon quantum dots as electron relay mediator then via carbon nanohorns to the electrode surface based on suitable band alignment. This hierarchical charge transport cascade paradigm efficiently impeded the charge recombination, resulting in noticeably increased photocurrent. In this competitive immunosensor, the AFB₁ standards compete with the labeled AFB₁, leading to photocurrent decreased with the increasing target concentration in a wide linear range of 10⁻⁶-10² ng/mL. The newly developed methodology provided a versatile approach for ultrasensitive detection of small molecules.

Keywords: Photoelectrochemical biosensor; Carbon quantum dots; TiO₂ mesocrystal; Si phthalocyanines; Aflatoxin B₁

Introduction

Mycotoxins, toxic secondary metabolites generated from fungal species, present in extensive range of foodstuff and feed product [1], which have detrimental effects, such as mutagenicity, immune suppression and hepatocellular carcinoma, on humans' health [2]. Aflatoxin B₁ (AFB₁) is the most frequently found mycotoxin and highly toxic one among the aflatoxins [3], which is ranked as group I carcinogen by the International Agency for Research on Cancer [4]. To monitor the contamination levels of AFB₁, a host of researches have been performed, such as electrochemical assay [5], enzyme-linked immunosorbent assay (ELISA) [6], UHPLC-MS/MS [7], electrochemiluminescent (ECL) [8] and Förster resonance energy transfer (FRET) [9] etc. Nevertheless, it is still urgent and critical to develop validated sensing techniques for rapid, accurate, and cost effective detection of AFB₁ at low concentration levels. The newly emerged photoelectrochemical (PEC) protocol with tremendous potential could be regarded as the next-generation detection platform owing to its high sensitivity, short response time, and simple apparatus [10]. This promising analytical methodology that based on the connection between the change of photocurrent and target concentration has become a powerful passport in the fields of food security, environmental monitoring, and clinical diagnostics [11]. Typically, the method commonly consists of sandwich-type and competitive-type PEC assay formats. Although the high specificity and sensitivity of sandwich-type detection platform, there exist several drawbacks, such as the lower photo-absorption rate, longer incubation time and more chemicals consumption due to the utilization of the two matched antibodies [6]. Besides, for the small molecules examining, the

competitive-type matrix could offer a more appreciative pattern, since target analyte would be monitored by virtue of its capability to compete with the labeled antigen/hapten [6, 12]. Conventionally, the detectable response mainly stems from the labeled enzyme that executes the oxidation or reduction reaction towards substrate to construct the probe components. Nevertheless, there exist some disadvantages of enzyme labeling, such as the unsatisfactory reproducibility, high cost of sensor fabrication, and instability for long-term experiment or clinical practice. One promising approach is to synthesis an effective tag that substitute enzyme to bypass these drawbacks and expanding the PEC immunoassays [13]. Up to present, the prevalent used photoactive materials are predominantly based on n-type inorganic semiconductor nanospecies [14, 15] or quantum dots [16-18], and the TiO_2 has turned out to be the prime candidate due to its multitudinous superiority, for example, the fine biocompatibility, eminent photoelectric activity, and environmental safety [19]. To further elevate the substantial light harvesting ability and hinder photo-excited electron-hole (e^-h^+) charge carriers recombination of TiO_2 (large band gap: ~ 3.2 eV), heterojunction construction and coupling with narrow-band semiconductor were commonly utilized synthetic strategies. Phthalocyanines (Pcs) are considered as efficient photo-sensitizer to harvest light on surface of the semiconductor and have become a research hotspot in frontier areas, for instance, dye-sensitized solar cells [20], electrochromic and electroluminescent displays [21], non-linear optics [22], photovoltaic and semiconductors [23] etc. The metalloid silicon (IV) Pcs (SiPcs, a p-type semiconductor) are a particularly attractive subclass of Pcs given the low toxicity levels of silicon and their low band gap (~ 1.7 eV) [24]. Additionally, SiPcs play an important role in developing solar light photocatalysts owing to their excellent electronic and optical properties, especially the unique absorption in red and near

infrared regions and strong electron-donating character [25]. However, the hydrophobic nature of Pcs rings lead to strong tendency to aggregation in solution and limiting their applications [26]. The more efficient way to suppress the aggregation of SiPcs is to attach dendritic substituents to the axial position of silicon centre [27]. This dendrimer SiPcs not only prevent self-aggregation, but also enhance the electron transfer efficiency. Herein, a facile strategy has been developed to intimately integrate quasi-octahedral TiO_2 mesocrystal (QOTM) with dendritic SiPcs as the signal amplification label (QOTM@SiPcs). The mesoporous QOTM could directly composite with the filling guest material of the dendritic SiPcs due to the enlarged contact area. By combining a p-type material with an n-type semiconductor, the resulting p/n heterostructure perform efficient visible-light absorption and charge separation, presenting an eminent probe tag for signal amplification.

Apart from the exquisite probe design in PEC sensing platform, the basal transducer scaffold construction is also critical for bioprocessing applications and obtaining favourable photocurrent response. In this paper, a new-type all-carbon nanohybrids sensitization (all-CNS) electrode system was developed based on the synergistic interaction between carbon quantum dots (CQDs) and carbon nanohorns (CNHs) to facilitate heterogeneous electron-transfer processes. Recently, fluorescent CQDs have been attracted countless attention, however, the investigations on PEC properties and applications are still rare. Compared with two-dimensional zero bandgap graphene, the 0-dimensional CQDs possess an intrinsic bandgap for photochemical reaction because of their higher optical activities associated with quantum confinement, edge effects [28] and visible light response [29, 30]. Therefore, CQDs-based sensor would be an innovative and meritorious report on photoelectric properties contrary to the traditional fluorescence (FL) sensing approaches. To further

improve the photocurrent response, the CNHs with high conductivity could receive the photo-induced electrons from the conduction band (CB) of CQDs to accelerate the electrons transport. The present work revealed that the integration of all-CNS materials provided a favourable model architecture for constructing PEC biosensing scaffold.

In this protocol, based on the specific recognition between antibody and antigen, an ultrasensitive PEC immunoassay for toxin monitoring was designed and the fabrication process was presented in Scheme 1A. Initially, the clean electrode was successively modified with CNHs, carboxymethyl chitosan (CMC) and CQDs to form the all-CNS materials modified electrode. Then the $-\text{COOH}$ of CMC was activated for following AFB_1 antibody (anti- AFB_1) anchoring. Subsequently, the electrode was immersed into the mixed solution containing AFB_1 and QOTM@SiPcs labeled AFB_1 ($\text{AFB}_1\text{-QOTM@SiPcs}$) conjugates to facilitate the competitive immune reaction. Therefore, a “signal-off” PEC platform was fabricated. With the increasing concentration of AFB_1 , the amount of $\text{AFB}_1\text{-QOTM@SiPcs}$ decreased and the photocurrent response reducing, thus indicating the AFB_1 content. The high sensitivity of this immunoassay mainly resulted from the multistep cascade electron transfer. Elaborately, QOTM@SiPcs conjugates could dramatically enhance the photocurrent derived from the increased light absorption of SiPcs as well as the effective electrons transfer from the lowest unoccupied molecular orbital (LUMO) of SiPcs to the CB of QOTM. Subsequently, the electrons could further arrive at the CB of CQDs due to the effective matching of energy levels between CQDs and QOTM, and then transferred immediately to CNHs, which resulted in enhanced charge separation and an obviously improved photocurrent response. The designed PEC competition immunoassay presented ultrahigh sensitivity, good reproducibility,

stability, and specificity, which was an efficient scheme for other small molecule determination.

2. Experimental

2.1 Materials and Reagents

AFB₁, monoclonal anti-AFB₁ were acquired from Sigma-Aldrich (Milwaukee, WI, USA). Bovine serum albumin (BSA) and CMC were purchased from Aipoo-Huamei Co., Ltd. (Beijing, China) and Maya bioengineering Co., Ltd. (Shanghai, China). Glutaraldehyde (GLD) and N, N-dimethylformamide (DMF) were obtained from Sinopham Chemical Reagent Co., Ltd. (Shanghai, China). The 4-aminothiophenol (4-ATP) was acquired from Acros Organics Chemical Co., Ltd. (China). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich. Phosphate buffer solution (PBS) was obtained by mixing stock solution of 0.1 M NaH₂PO₄ and 0.1 M Na₂HPO₄ to adjust the pH. Other reagents were of analytical reagent grade. CNHs and CQDs (10 mg/mL) were prepared with DMF for future use.

2.2 Synthesis of bis-pentyl-carboxylate SiPcs and the preparation of AFB₁-QOTM@SiPcs bioconjugates

The synthesis procedure of bis-3-methyl-butoxy SiPcs was described briefly below. Initially, SiPcCl₂ (0.20 g, 0.33 mM) with anhydrous K₂CO₃ (0.14 g, 1.01 mM) and hexanoic acid (0.5 mL, 3.99 mM) in dried toluene (30 mL) was refluxed at 110 °C for 48 h. The reaction mixture was then cooled to room temperature and the solvent was evaporated to dryness under reduced pressure. The solid product was purified for 24 h by soxhlet extraction using dichloromethane (DCM) as an extracting agent. The extracted material was recrystallized, washed with methanol and deionized (DI) water (v:v=1:1), and then re-dissolved in DCM. The AFB₁-QOTM@SiPcs

bio-conjugates were prepared as followings. Initially, the synthetic dendritic SiPcs (30 μL) was added in 10 μL of 10 mg/L QOTM for 1h to allow the sufficient adsorption, and the unconjugated SiPcs were removed by centrifugation and washed with DI water. Thereafter, 20 μL of 0.1 mmol/L 4-ATP was dropped onto the mixed solution containing QOTM@SiPcs conjugates and incubated for 4 h. Following that, the unbounded 4-ATP was removed by centrifugation and washed with DI water. Finally, 40 μL of 10 ng/mL AFB₁ was added in the resulting solution for 3h, and then centrifuged and washed by DI water. With the aid of 4-ATP, QOTM@SiPcs could be labeled onto the AFB₁ due to the formation of a strong bond (Ti-S) between the thiol end of 4-ATP molecule and QOTM, and the remaining amino group could form an imine bond with AFB₁.

2.3 Preparation of all-CNS materials modified electrode

Firstly, 3 μL of 3 mg mL^{-1} CNHs were coated on the clean surface of glassy carbon electrode (GCE; 3 mm in diameter), and then placed under an IR lamp until the solvents were evaporated and cooled to room temperature. Afterwards, 3 μL CMC (0.5 wt %) was thrown on the CNHs modified electrode for 40 min to allow the reaction between carboxyl group (-COOH) at conical terminals of CNHs [31] and hydroxyl (-OH) of CMC. And then the electrode was washed with ultrapure water and dried at room temperature to avoid the exfoliation of CNHs. Sequentially, CQDs were deposited on the electrode, dried in the IR lamp and cooled down to room temperature, and the all-CNS materials modified electrode was fabricated.

2.4 Construction of the competitive PEC sensor for AFB₁

Concisely, anti-AFB₁ (5 μL , 3.0 μM) was immobilized on the all-CNS materials modified electrode surface using EDC/NHS as cross-linking. In short, 10 μL of EDC (10 mM) and NHS (20 mM) aqueous solution was dropped onto the

GCE/CNHs/CQDs electrode surface to active the –COOH group of CMC, and kept in dark for 1 h at 4 °C, followed by anchoring anti-AFB₁. After being dried, the obtained electrode was coated with BSA (5 µL, 1.0 wt %) at 4 °C for 1 h. Finally, the electrode was immersed into the mixed solution containing AFB₁ standard solution and QOTM@SiPcs labeled AFB₁ to facilitate the competition. The photocurrent was measured in pH 8.0 PBS at the applied potential of 0 V. After each step, the sensor was washed with DI water.

2.5 Preparation of peanut samples

The fresh peanut was purchased from a local market of agriculture products and ground finely with a laboratory mill. Subsequently, 6 g of sample was placed in a 50 mL centrifuge tube, and 18 mL of methanol/water (80:20, v/v) containing 4% NaCl was added [32] to facilitate the sample to be extracted via vortex mixing. After centrifugation at 4000 rpm for 5 min, 1.2 mL of supernatant was transferred to a 3.0 mL tube and diluted with 1.8 mL of pure water. The resulting solution was used as blank sample for this analytical method.

3. Results and Discussion

3.1 Morphology and composition characterization

To get insight into the morphologies and structures of the QOTM, typical scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images were provided. As shown in Fig. 1A, the size of crystallites with rough surface was 25-50 nm, and the octahedral was built by monodisperse nanoparticles subunits to construct the porosity structure. By using QOTM with large surface area, more dendritic SiPcs molecules could fill into the inner portion of the mesoporous QOTM, forming a close contact structure with TiO₂ mesocrystal. To further investigate the organization of anatase TiO₂ nanostructure, TEM (inset in Fig. 1A) was performed. It

could be observed that the quasi-octahedral shape was constructed from very tiny nanoparticles, which was corresponding to the results of SEM image. Herein, the unique-ordered TiO₂ quasi-octahedral was introduced as photo-sensitizer element in this PEC determination.

The UV/vis spectra of dendrimer SiPcs with terminal groups in DMF are shown in Fig. 2B, which presented typical spectra for Pcs with a B band at 355 nm, a dimer band at 604 nm, and a Q band at 678 nm. Additionally, a UV band ranging from 251-292 nm could be attributed to the absorption of dendritic terminal groups substitutes. Compared with Q band of non-dendrimer SiPcCl₂ at 670 nm [26], the Q bands of SiPcs with dendritic substitutes were red-shifted 8 nm. This outcome demonstrated that the substituent group linked with Si atom via ether bonds could form expanded π conjugation system, thus diminishing the electronic density of Pcs ring and the energy level gap between LUMO and highest occupied molecular orbital (HOMO). The inset of Fig. 2B showed the position and intensity of fluorescence (FL) spectra of bis-pentyl-carboxylate SiPcs. The maximum FL emission peak was observed at 694 nm, which was in agreement with the perception that electron-withdrawing terminal groups enhanced the rigidity of molecular, reducing the molecular internal conversion.

The topography of CQDs was monitored using TEM (Fig. 1C). The particles exhibited a relatively narrow size distribution and spherical symmetry, with an average diameter of 2-3 nm. Additionally, the optical properties of CQDs were tested, which was indicated in Fig. 1D. The UV-vis spectra of aqueous exhibited a broad absorption from 300 to 600 nm. Such a high absorbance was conducive for generation of photocurrent under light irradiation. The FL spectra exhibited the emission at ~529 nm when excited at 368 nm, originating from the excited electrons returning to

ground state and recombined with holes.

3.2 Excellent PEC performance of QOTM@SiPcs

Pcs and its derivatives are promising sensitized materials for photochemical energy conversion and storage owing to their excellent light-harvesting properties in the red/near-IR (Q band) regions [24]. Therefore, in this work, Pcs were utilized as sensitizer for wide-bandgap oxide semiconductor. In order to conquer the low efficiency derived from its aggregation and lack of directionality in the excited state, an intriguing symmetrical SiPcs sensitizer containing bis-pentyl-carboxylate were acted as “push” group was designed. The purpose of the bis-pentyl-carboxylate was to minimize the aggregation, enhance solubility, and tune the LUMO level of SiPcs that offered directionality in the excited state. Therefore, the dendritic SiPcs was introduced here to combine with QOTM for acquiring excellent photoelectric conversion efficiency, and a series of PEC measurements were performed to prove this viewpoint. Initially, as shown in Fig. 2A, QOTM@SiPcs presented a larger photocurrent density in contrast with QOTM under the applied potentials from 0.0-0.6 V vs Ag/AgCl at a scan rate of 5 mV/s with chopped light irradiation, indicating that QOTM@SiPcs owned a better PEC performance and the dendritic SiPcs could enhance the light absorption and trigger the charge-carrier separation.

The incident photon-to-current conversion efficiency (IPCE) plots were the other fundamental means to illustrate the photoelectric behaviours of the photoactive materials by measuring IPCE values at different wavelengths. The outcome presented in Fig. 2B was according to Eq. (1) [33] with no bias voltage.

$$IPCE\% = \frac{1240 \times j}{\lambda \times P} \times 100 \quad (1)$$

Where P, λ and j were the incident light power in W cm^{-2} , wavelength in nm, and

short circuit photocurrent density in A cm^{-2} . Evidently, an elevated IPCE value was observed in whole spectral range for QOTM@SiPcs by contrast to the pristine QOTM, which could be brought to the heterojunction construction that caused wide light absorption and quick electrons transfer. Additionally, it was intriguing to note that the maximum IPCE value associated with the corresponding wavelength for QOTM@SiPcs had a phenomenon of red-shift, further expanding the utilization of optical scope.

Subsequently, Fig. 2C of the typical open-circuit photovoltage (V_{oc}) response was employed from another perspective to elucidate the prominent character of QOTM@SiPcs. Under open circuit condition, the inspired photoelectrons transferred and accumulated within the nanostructure films upon light illumination, causing a more negative potentials shift of the Fermi level and an increase in open-circuit voltage [34]. Accompanied with the competition between charge recombination and photoelectron accumulation, the V_{oc} achieved to a stable state (maximum value) under light irradiation. Once light was stopping, the V_{oc} decayed as the collected electrons were relaxed owing to the recombination with photo-generated holes and the scavenging by electron acceptor species in electrolyte [35]. The significant drop in V_{oc} and slowing decay observed with QOTM@SiPcs indicated that more photo-induced electrons in QOTM@SiPcs were generated and survived longer.

According to this model, the decay lifetime of accumulated electrons could be related to the reduce in potential using Eq. 2 [36].

$$\tau = \frac{k_B T}{e} \left(\frac{dV_{oc}}{dt} \right)^{-1} \quad (2)$$

Where τ , e , and $k_B T$ were the potential dependent lifetime, charge of a single electron, and the thermal energy in which k_B was Boltzmann's constant and T was temperature in K. The dV_{oc}/dt was the derivative of open-circuit voltage transient. The

relationship between electrons lifetime and V_{oc} in QOTM and QOTM@SiPcs was presented in Fig. 2D. Obviously, QOTM@SiPcs exhibited a longer decay lifetime of the accumulated electrons. These comparison experiments sufficiently demonstrated the admirable photoelectric performance of dendritic SiPcs sensitized QOTM.

3.3 EIS and PEC characterization of the biosensor

Electrochemical impedance spectroscopy (EIS) was an available way to characterize interface properties and construction procedures of the electrodes. The electron transfer resistance (R_{et}) was equal to the semicircle diameter at low frequency, which represented the charge transfer resistance of redox probe accessing the layer [11]. As depicted in Fig. 3A, the electrode of GCE/CNHs had a smaller semicircle (curve b) in comparison with bare GCE (curve a), indicating the prominent conductivity for CNHs. After the CQDs were assembled onto the GCE/CNHs electrode, the R_{et} increased (curve c), implying the formation of new film hindered the access of redox probe to the electrode surface. After gradual immobilization of anti-AFB₁ (curve d), the R_{et} was enlarged, owing to the protein layer modified PEC sensing matrix hindered electron transfer of the electrochemical probe to the electrode surface. The semicircle diameter was further increased when AFB₁ anchored on the electrode (curve e), suggesting the successful assembling of AFB₁. However, the electrode of GCE/CNHs/CQDs/anti-AFB₁ incubated with AFB₁-QOTM@SiPcs possessed higher electron-transfer resistance, proving the weak conductivity of QOTM@SiPcs and the conjugates was successfully fabricated.

Except for the investigation of the construction procedures, the photocurrent response of different modified electrodes was explored to certify the feasibility of this biosensor. As displayed in Fig. 3B, the photo-responses increased gradually along with the CQDs (curve b) and CNHs/CQDs (curve c) respectively modified electrode,

which revealing that the CQDs owned moderate photocurrent response and the CNHs could efficiently improve the photoelectric performance for CQDs due to their pronounced conductivity. Therefore, this all-CNS photoactive species could be applied as the PEC matrix for basal signal source as well as the scaffold for following immune reaction. The photo-responses declined after immobilization of anti-AFB₁ and AFB₁, which was attributed to the formation of hydrophobic multilayers that hampered interfacial mass and electron transfer. Nevertheless, the AFB₁-QOTM@SiPcs modified electrode showed remarkable enhancement of photocurrent, indicating the successful combination of the tag with the corresponding labeling AFB₁. Meanwhile, this phenomenon manifested that the heterostructures could finely promote the photoresponse and this signal amplification strategy afforded a general format for future development of PEC assays.

3.4 The PEC mechanism for the AFB₁ detection

Constructing an efficient and delicate bioprobe has a substantial influence on whether the signal to be measured would be sensitive and accurate while the amounts of target are changed. Herein, an advantageous enzyme-free tag comprised of QOTM@SiPcs conjunction was designed and utilized for labeling AFB₁ antigen. In comparison with the photoelectric response of pristine QOTM (curve a in Fig. 4A), dendritic SiPcs sensitized QOTM owned an excellent photoresponse (curve b in Fig. 4A) that was two times higher than QOTM. To further certify the feasibility of SiPcs sensitization when applied in the AFB₁ monitoring, the other comparison experiment was performed to elucidate the applicability of dendritic SiPcs. As displayed in Fig. 4B, both QOTM (curve b) and QOTM@SiPcs (curve c) labeled AFB₁ enlarged photocurrent response compared with the untagged AFB₁ (curve a). Nevertheless, from the histogram inserted in Fig. 4B, a remarkable contrast could be observed that

the amplification deviation for QOTM@SiPcs (blue) was larger than QOTM (pink), which confirmed that dendritic SiPcs was a viable option in this biosensor.

Additionally, the PEC matrix plays a vital part in enhancing the basal response to avoid the immune reaction process that causes the detection signal to vanish or fade away. Therefore, a fancy all-CNS structure was employed as the photoelectrode scaffold, in which the CNHs substrate with high conductivity could accelerate the electrons transfer and enhance the photocurrent response of CQDs (a series of PEC measurements were performed to verify the sensitization of CNHs for CQDs, please see Fig. S1 in supporting information). Based on the eminent construction of bioprobe and PEC matrix design, a versatile competitive-type immunoassay was established, which connect these two basilica parts through the immune traction. The photo-initiated electrons transfer process of the sensor was shown in Scheme 1B. Under irradiation, photo-generated electrons from the LUMO level of SiPcs could be migrated to QOTM. For the all-CNS structure, CQDs could be acted as a bridging medium to reinforce the flow of electrons that receiving them from the CB of QOTM, therefore increasing the density of photo-generated electrons (carve c in Fig. 4A). Finally, the electrons transferred to the external circuit with the assistance of conductive CNHs, simultaneously improved the photocurrent response (carve d in Fig. 4A). Dendritic SiPcs, QOTM, CQDs, and CNHs presented a multistep cascade band-edge level, which promoted ultrafast transfer of e^-h^+ pairs and inhibited charge recombination. In short, this methodology provided an available work for practical use in quantitative small molecular assay for life and food safety.

3.5 Optimization of the PEC biosensing platform and experimental conditions

To achieve better analytical performance, the condition of the biosensing platform construction including the amounts of CNHs, CQDs, absorption time of

SiPcs on QOTM, and the ratio between QOTM and AFB₁ for construction of the bioprobe were measured. The optimal usage quantity was 3 mg/mL for both CQDs and CNHs (Fig. S2A and S2B). As for SiPcs, the absorption time was 1h, which receiving optimal photoelectric response (Fig. S2C). Certainly, the conjugated amounts of QOTM@SiPcs and AFB₁ also affect the sensitivity of PEC sensor, and an optimal signal could be acquired at a 2:5 volume ratio of 0.5 mg/mL QOTM and 100 ng/mL AFB₁ (the total volume was 100 μ L). Additionally, several experimental parameters such as anti-AFB₁ and AFB₁ incubation time, pH value, and applied potential were also investigated (Fig. S3). Incubation time for anti-AFB₁ and AFB₁-QOTM@SiPcs were both 1 h and the detection measurements were performed in PBS of pH 8.0 and applied potential of 0 V. The detailed explanation was submitted in supporting information.

3.6 Analytical performance of the signal-off biosensor toward the target AFB₁

To evaluate the detect-ability of the PEC bioassay, AFB₁ standards with different concentrations were tested on the basis of all-CNS structure accompanied with an efficient signal amplification tag of QOTM@SiPcs for altering the signal change under the optimal conditions. The assay was carried out by using amperometric I-t curves in pH 8.0 PBS. As displayed in Fig. 5A, the degree of signal reduction was directly related to the increasing AFB₁ concentration. A linear correlation (shown in Fig. 5B) between the photocurrent density and the logarithm of AFB₁ concentration was obtained in the dynamic range of 10⁻⁶-10² ng/mL with a detection limit (LOD) of 3.2 $\times$ 10⁻⁷ ng/mL. Appreciatively, the detection range was wider and the LOD was lower in comparison with other reported previously (Table S1). The regression equation could be fitted to j (μ A cm⁻²) = -0.17464 log C (ng/mL)+1.73972 with R²=0.9959. Additionally, the feasibility and practicability of this strategy was

investigated on real sample of peanuts with recovery rate of 96.8-103.1% (Table S2).

3.7 Specificity, reproducibility, and stability of the PEC biosensor

Specificity was critical for most of the bioassays, because nonspecific binding would mislead the detection outcomes. The selectivity of the fabricated PEC biosensor was estimated by incubating some interfering antigens including zearalenone (Zea), fumonisins (Fum), streptomycin (Str), and geomycin (Geo) with an excess hundred folds concentration of AFB₁ as well as the mixtures of these interferences with AFB₁. As shown in Fig. 5C, the detection signal of these interferences was lower than AFB₁ and all mixtures had no apparent influence on AFB₁ detection, demonstrating the present multiple amplifying biosensor possessed fine specificity.

The reproducibility of the proposed bioassay was assessed by relative standard deviations (RSDs) of both intra-assay and inter-assay. Calculated from the detection results with twelve parallel tests and the RSDs of intra-assay were 2.8%, 3.2%, and 3.6% toward 0.01, 1.0, and 100 ng/mL of target AFB₁, respectively. The inter-assay RSDs of 4.6%, 5.2%, and 3.8% were obtained by testing the identical samples with twelve biosensors prepared separately at the same experimental condition. These results revealed a commendable reproducibility and satisfactory precision.

The stability of the biosensor was investigated and no obvious change of photocurrent response could be observed within 450 s under interrupted light irradiations in Fig. 5D, indicating a good stability of the PEC assay. In addition, the biosensing electrode of GCE/CNH/CQD/anti-AFB₁/AFB₁-QOTM@SiPcs was stored in a dark and humid environment at 4 °C for 2 weeks, the photocurrent intensity still retained 94.5% of its starting value, revealing its good long-term storage stability.

Conclusions

In summary, a competitive-type PEC immunoassay for ultrasensitive detection of toxic AFB₁ was established based on the cooperation effect of all-CNS structure and PEC bioprobe of QOTM@SiPcs conjugates. As PEC scaffold, the all-CNS structure could noticeably enhance photocurrent owing to admirable properties of absorption of illuminating light, ultrafast transfer of electrons, and efficient suppression of charge recombination. Bioconjugates of AFB₁ and QOTM@SiPcs was introduced by specific immune reaction, and the QOTM@SiPcs conjugates could dramatically enhance photocurrent response due to high absorption of exciting light of this heterojunction. More importantly, because of the effective matching of energy levels between SiPcs, QOTM, and CQDs as well as the fast electron migration of the CNHs, the sensing platform showed amplified photocurrent intensity. This protocol not only could be extended to analyse a diverse range of small molecules by using corresponding affinity ligand, but also offered new horizons for providing a reliable and intuitionistic guidance for the development of bioanalytical methods.

Acknowledgements

This project was financially supported by the NSFC (21205016, 21575024), National Science Foundation of Fujian Province (2016J06003, 2016J05026), Education Department of Fujian Province (JA14071, JK2016009, JA13068) and Foundation of Fuzhou Science and Technology Bureau (2015-S-160, 2015-G-72) and New Century Talent Project of Fujian Province. Authors also thanks to the technical help from Shenzhen Key Laboratory of Ultrahigh Refractive Structural Material (CXB201105100093A)

References

- [1] A.C. Mak, S.J. Osterfeld, H. Yu, S.X. Wang, R.W. Davis, O.A. Jejelowo, N. Pourmand, Sensitive giant magnetoresistive-based immunoassay for multiplex mycotoxin detection, *Biosens. Bioelectron.* 25 (2010) 1635-1639.
- [2] X. Li, P.W. Li, Q. Zhang, R. Li, W. Zhang, Z.W. Zhang, X.X. Ding, X.Q. Tang, Multi-component immunochromatographic assay for simultaneous detection of aflatoxin B₁, ochratoxin A and zearalenone in agro-food, *Biosens. Bioelectron.* 49 (2013) 426-432.
- [3] D. Wang, W. Hu, Y. Xiong, Y. Xu, C.M. Li, Multifunctionalized reduced graphene oxide-doped polypyrrole/pyrrolepropylic acid nanocomposite impedimetric immunosensor to ultra-sensitively detect small molecular aflatoxin B₁, *Biosens. Bioelectron.* 63 (2015) 185-189.
- [4] A. Li, L. Tang, D. Song, S. Song, W. Ma, L. Xu, H. Kuang, X. Wu, L. Liu, X. Chen, C. Xu, A SERS-active sensor based on heterogeneous gold nanostar core-silver nanoparticle satellite assemblies for ultrasensitive detection of aflatoxinB₁, *Nanoscale* 8 (2016) 1873-1878.
- [5] Y. Lin, Q. Zhou, Y. Lin, D. Tang, R. Niessner, D. Knopp, Enzymatic hydrolysate-induced displacement reaction with multifunctional silica beads doped

with horseradish peroxidase-thionine conjugate for ultrasensitive electrochemical immunoassay, *Anal. Chem.* 87 (2015) 8531-8540.

[6] F. Y. Yu, A. V. Gribas, M. M. Vdovenko, I. Y. Sakharov, Development of ultrasensitive direct chemiluminescent enzyme immunoassay for determination of aflatoxin B₁ in food products, *Talanta* 107 (2013) 25-29.

[7] N. Zitomer, M.E. Rybak, Z. Li, M.J. Walters, M.R. Holman, Determination of aflatoxin B₁ in smokeless tobacco products by use of UHPLC-MS/MS, *J. Agric. Food. Chem.* 63 (2015) 9131-9138.

[8] G. Xu, S. Zhang, Q. Zhang, L. Gong, H. Dai, Y. Lin, Magnetic functionalized electrospun nanofibers for magnetically controlled ultrasensitive label-free electrochemiluminescent immune detection of aflatoxin B₁, *Sens. Actuators, B* 222 (2016) 707-713.

[9] W. Xu, Y. Xiong, W. Lai, Y. Xu, C. Li, M. Xie, A homogeneous immunosensor for AFB₁ detection based on FRET between different-sized quantum dots, *Biosens. Bioelectron.* 56 (2014) 144-150.

[10] N. Zhang, Z.Y. Ma, Y.F. Ruan, W.W. Zhao, J.J. Xu, H.Y. Chen, Simultaneous photoelectrochemical immunoassay of dual cardiac markers using specific enzyme tags: a proof of principle for multiplexed bioanalysis, *Anal. Chem.* 88 (2016) 1990-1994.

[11] J. Shu, Z. Qiu, Q. Zhou, Y. Lin, M. Lu, D. Tang, Enzymatic oxydate-triggered self-illuminated photoelectrochemical sensing platform for portable immunoassay using digital multimeter, *Anal. Chem.* 88 (2016) 2958-2966.

- [12] M.H. Kim, S.J. Choi, Immunoassay of paralytic shellfish toxins by moving magnetic particles in a stationary liquid-phase lab-on-a-chip, *Biosens. Bioelectron.* 66 (2015) 136-140.
- [13] J. Song, J. Wang, X. Wang, W. Zhao, Y. Zhao, S. Wu, Z. Gao, J. Yuan, C. Meng, Using silver nanocluster/graphene nanocomposite to enhance photoelectrochemical activity of CdS:Mn/TiO₂ for highly sensitive signal-on immunoassay, *Biosens. Bioelectron.* 80 (2016) 614-620.
- [14] Z. Kang, Y. Gu, X. Yan, Z. Bai, Y. Liu, S. Liu, X. Zhang, Z. Zhang, X. Zhang, Y. Zhang, Enhanced photoelectrochemical property of ZnO nanorods array synthesized on reduced graphene oxide for self-powered biosensing application, *Biosens. Bioelectron.* 64 (2015) 499-504.
- [15] J. Zhuang, D. Tang, W. Lai, M. Xu, D. Tang, Target-induced nano-enzyme reactor mediated hole-trapping for high-throughput immunoassay based on a split-type photoelectrochemical detection strategy, *Anal. Chem.* 87 (2015) 9473-9480.
- [16] G.C. Fan, L. Han, J.R. Zhang, J.J. Zhu, Enhanced photoelectrochemical strategy for ultrasensitive DNA detection based on two different sizes of CdTe quantum dots cosensitized TiO₂/CdS:nin hybrid structure, *Anal. Chem.* 86 (2014) 10877-10884.
- [17] X. Zeng, S. Ma, J. Bao, W. Tu, Z. Dai, Using Graphene-based plasmonic nanocomposites to quench energy from quantum dots for signal-on photoelectrochemical aptasensing, *Anal. Chem.* 85 (2013) 11720-11724.
- [18] W.W. Zhao, J. Wang, Y.C. Zhu, J.J. Xu, H.Y. Chen, Quantum dots: electrochemiluminescent and photoelectrochemical bioanalysis, *Anal. Chem.* 87

(2015) 9520-9531.

[19] J. Shu, Z. Qiu, J. Zhuang, M. Xu, D. Tang, In situ generation of electron donor to assist signal amplification on porphyrin-sensitized titanium dioxide nanostructures for ultrasensitive photoelectrochemical immunoassay, *ACS Appl. Mater. Interfaces* 7 (2015) 23812-23818.

[20] B. Lim, G.Y. Margulis, J.H. Yum, E.L. Unger, B.E. Hardin, M. Grätzel, M.D. McGehee, A. Sellinger, Silicon-naphthalo/phthalocyanine-hybrid sensitizer for efficient red response in dye-sensitized solar cells, *Org. Lett.* 15 (2013) 784-787.

[21] M.L. Rodriguez-Mendez, R. Aroca, J.A. DeSaja, Electrochromic and gas adsorption properties of Langmuir-Blodgett films of lutetium bisphthalocyanine complexes, *Chem. Mater.* 5 (1993) 933-937.

[22] H.A. Abdeldayem, D.O. Frazier, B.G. Penn, D.D. Smith, C.E. Banks, Non-linear optothermal properties of metal-free phthalocyanine, *Thin Solid Films* 350 (1999) 245-248.

[23] B.H. Lessard, J.D. Dang, T.M. Grant, D. Gao, D.S. Seferos, T.P. Bender, Bis(tri-n-hexylsilyl oxide) silicon phthalocyanine: a unique additive in ternary bulk heterojunction organic photovoltaic devices, *ACS Appl. Mater. Interfaces* 6 (2014) 15040-15051.

[24] E. Zysman-Colman, S.S. Ghosh, G. Xie, S. Varghese, M. Chowdhury, N. Sharma, D.B. Cordes, A.M.Z. Slawin, I.D.W. Samuel, Solution-processable silicon phthalocyanines in electroluminescent and photovoltaic devices, *ACS Appl. Mater. Interfaces* 8 (2016) 9247-9253.

- [25] J. Huang, Y. Wu, D. Wang, Y. Ma, Z. Yue, Y. Lu, M. Zhang, Z. Zhang, P. Yang, Silicon phthalocyanine covalently functionalized n-doped ultrasmall reduced graphene oxide decorated with pt nanoparticles for hydrogen evolution from water, *ACS Appl. Mater. Interfaces* 7 (2015) 3732-3741.
- [26] H. Başı, Z. Biyiklioglu, Non-aggregated axially naphthoxazin group substituted silicon phthalocyanines: Synthesis and electrochemistry, *J. Organomet. Chem.* 791 (2015) 238-243.
- [27] D. Ma, S. Pan, T. Zhang, B. Huang, S. Xie, H. Yang, Y. Peng, Comparison of multiple terminal functional groups dendrimer silicon(IV) phthalocyanines: Photoinduced electron/energy transfer and electrochemical properties, *Dyes Pigm.* 127 (2016) 78-86.
- [28] Q. Liu, B. Guo, Z. Rao, B. Zhang, J.R. Gong, Strong two-photon-induced fluorescence from photostable, biocompatible nitrogen-doped graphene quantum dots for cellular and deep-tissue imaging, *Nano Lett.* 13 (2013) 2436-2441.
- [29] X. Yu, J. Liu, Y. Yu, S. Zuo, B. Li, Preparation and visible light photocatalytic activity of carbon quantum dots/TiO₂ nanosheet composites, *Carbon* 68 (2014) 718-724.
- [30] N.N. Chai, H.X. Wang, C.X. Hu, Q. Wang, H.L. Zhang, Well-controlled layer-by-layer assembly of carbon dot/CdS heterojunctions for efficient visible-light-driven photocatalysis, *J. Mater. Chem. A* 3 (2015) 16613-16620.
- [31] S. Zhu, G. Xu, Single-walled carbon nanohorns and their applications, *Nanoscale* 2 (2010) 2538-2549.

- [32] X. Xu, X. Liu, Y. Li, Y. Ying, A simple and rapid optical biosensor for detection of aflatoxin B1 based on competitive dispersion of gold nanorods, *Biosens. Bioelectron.* 47 (2013) 361-367.
- [33] A. Kongkanand, K. Tvrđy, K. Takechi, M. Kuno, P.V. Kamat, Quantum dot solar cells. tuning photoresponse through size and shape control of CdSe-TiO₂ architecture, *J. Am. Chem. Soc.* 130 (2008) 4007-4015.
- [34] Q. Chen, H. Liu, Y. Xin, X. Cheng, J. Li, Enhanced photoelectrochemical and photocatalytic performance of single-crystalline anatase TiO₂ (101) nanobelts arrays originating from nanotubes arrays, *Appl. Surf. Sci.* 264 (2013) 476-484.
- [35] X.M. Song, J.M. Wu, M.Z. Tang, B. Qi, M. Yan, Enhanced photoelectrochemical response of a composite titania thin film with single-crystalline rutile nanorods embedded in anatase aggregates, *J. Phys. Chem. C* 112 (2008) 19484-19492.
- [36] B.H. Meekins, P.V. Kamat, Got TiO₂ nanotubes? lithium ion intercalation can boost their photoelectrochemical performance, *ACS Nano* 3 (2009) 3437-3446.

Figure captions

Scheme 1 (A) Fabrication procedure of the PEC immunosensor and (B) the mechanism of the photo-initiated electron transfer pathways and the structure of dendritic SiPcs.

Fig. 1 (A) SEM, TEM (inset) and SAED (inset) images of QOTM, (B) UV-visible adsorption and FL (inset) spectra of dendritic SiPcs, (C) TEM image of CQDs, (D) UV-visible adsorption (a) and FL (b) spectra of CQDs.

Fig. 2 (A) Applied potential bias-dependent photocurrent densities, (B) IPCE spectra at applied potential of 0 V, (C) Voc time profile and (D) electron lifetimes measurements (determined from the Voc decay in dark) of (a) QOTM and (b) QOTM/SiPcs in 0.1M PBS (pH 8.0).

Fig. 3 (A) EIS spectra and PEC response of various modified electrodes. (a, a') GCE; (b) GCE/CNHs, (b') GCE/CQDs; (c, c') GCE/CNHs/CQDs; (d, d') GCE/CNHs/CQDs/anti-AFB1; (e, e') GCE/CNHs/CQDs/anti-AFB1/AFB1; (f, f') GCE/CNHs/CQDs/anti-AFB1/AFB1-QOTM@SiPcs. The concentration of AFB1 was 10 ng/mL. The EIS spectra were obtained in 5.0 mmol/L $[\text{Fe}(\text{CN})_6]^{3-/4-}$ solution containing 0.1 mol/L KCl. The PEC tests were performed in 0.1 M PBS (pH 8.0) under the applied potential of 0 V.

Fig. 4 (A) Photoactive species characterizations of (a) QOTM, (b) QOTM@SiPcs, (c) CQDs/QOTM@SiPcs, and (d) CNHs/CQDs/QOTM@SiPcs in 0.1 M PBS (pH 7.0). (B) Photocurrent responses of (a) CNHs/CQDs/anti-AFB₁/AFB₁, (b) CNHs/CQDs/anti-AFB₁/AFB₁-QOTM, (c) CNHs/CQDs/anti-AFB₁/AFB₁-QOTM@SiPcs in 0.1 M PBS (pH 8.0). The concentrations of anti-AFB₁ and AFB₁ were 3 µg/mL and 10 ng/mL, respectively. The inserts in Fig. 4B was the corresponding histogram comparison chart. All experiments were performed at 0 V applied potential.

Fig. 5 (A) The effect of different concentrations (a-h) 10⁻⁶-10² ng/mL AFB₁ on differential photocurrent density. (B) The corresponding calibration curve. (C) Selectivity of the immunoassay towards AFB₁ (0.1 ng/mL) determined by comparing with interfering agents (10 ng/mL) and corresponding mixture. (D) Time-based photocurrent density measured with light switched on and off toward 10⁻⁶ ng/mL AFB₁. The experiments were recorded at 0 V applied potential in 0.1 M PBS (pH 8.0).

Scheme 1

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5