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RESEARCH PAPER

One-step synthesized flower-like materials used for sensitively detecting amyloid precursor protein

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Abstract

In this paper, we developed a new method to detect Alzheimer's disease (AD)-related amyloid precursor protein (APP). A composite material containing horseradish peroxidase (HRP), APP antibody, and $Cu_3(PO_4)_2$ was synthesized as the biosensor by co-precipitation method. In this competitive immunoassay, APP was first conjugated onto the microplate surface with the help of poly-L-lysine as the coating reagent; the composite materials were then attached onto the microplate through the interaction of APP and antibody; the HRP can catalyze the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) and formed colored species. Therefore, the more APP in the detection solution (free form), the less composite material was combined with the immobilized APP on the microplate, resulting in the production of less colored TMB species. A series of detection parameters were studied, such as the composite material synthesis process, the concentration, and reaction time of different compounds. Our method has higher sensitivity compared with the similar immunoassay without using composite materials (the limits of detection are 0.3 and 3 ng/mL, respectively), and can be used for real samples (human serum) detection. The detection results using our method are consistent with the ELISA results, which is useful for the AD detection.

Keywords Competitive immunoassay · Amyloid precursor protein · Alzheimer's disease · Poly-L-lysine · ELISA

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative pathology that is the most common cause of dementia [1–3]. AD is characterized by deposition of extracellular amyloid plaque, the major component being the β-amyloid peptide (Aβ). Furthermore, Aβ is the enzymatic degradation product of amyloid precursor protein (APP) [4–7], and the over-production of APP is correlated with early-stage of AD. Nowadays, the risk of over-produced APP in the human body on the development of AD are often overlooked, especially if considering that AD is not reversible at middle or late stage when large amounts of Aβ is assembled [7, 8], and there is no effective drug to inhibit the production of Aβ or used to clear away the produced Aβ aggregates [3, 8–10]. Therefore, considering the close relationship between AD development and the amount of APP in body fluids [i.e., human plasma and cerebrospinal fluid (CSF)], sensitive and quantitative detection of APP in body fluids is helpful to develop a clinical diagnostic tool for AD [11–13]. Recently, numerous methods, including colorimetric assay [14], surface plasmon resonance [15], resonance light scattering [16], and dot-blot immunoassay [17] were developed to detect Aβ. Nevertheless, up to now, there is no report to detect APP with high sensitivity and selectivity for detecting AD progress, since AD is irreversible when Aβ was vastly produced at middle and advanced stages [18–20], and no curative pharmacological treatment for AD is available at present. It is necessary and urgent to develop a simple and practical method to monitor and interfere with the progress of AD at early-stage.

Recently, protein-inorganic composite flower-like materials containing proteins and $Cu_3(PO_4)_2$ have attracted much attention [21–24], since they are easy to prepare, have distinct hierarchical microstructures, have large active surface areas [25], have the ability to combine numerous antibodies and enzyme molecules (e.g., horseradish peroxidase, HRP) without sophisticated experimental procedures. They can, therefore, be used for the determination of different biomolecules with enhanced

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sensitivity [26–28]. As the composite functional materials possess large ratio of surface-to-volume, which facilitated the immobilization of lots of recognition molecules, e.g., antibodies, enzymes, or organic recognition molecules, these materials were widely used for the detection of glucose [24], H_2O_2[23], small organic molecules [28], even bacteria (e.g., Escherichia coli) [22], as well as for improving the interaction ability of compounds [25, 29]. On the other hand, in traditional enzyme-linked immunosorbent assay (ELISA), the attachment of coating reagents (i.e., antigen or antibody) is mainly based on the electrostatic and hydrophobic interactions between molecules and the surface of microplate. These weak interactions resulting in the abilities of small molecules binding onto the ELISA plates are relatively low [30, 31]. Therefore they hinder the performance of ELISA dramatically, and make the detection signals relatively low. It is important to improve the immobilization efficiencies of functional molecules on the microplates, and to enhance the signal-to-noise ratio, in order to detect target molecules with high sensitivity and selectivity. As there are numerous -NH_2 in poly-L-lysine (PLL) molecules, and high molecular weight compound may dramatically increase the electrostatic and hydrophobic interactions, glutaraldehyde could act as a good linker to link two -NH_2 containing molecules. Therefore, in recent reports, PLL with high molecular weight was applied to improve the immobilization efficiencies of molecules [31–34], and the glutaraldehyde-based bioconjugation technology was also used to improve the molecule conjugation ability and increase the coating efficiencies. In this paper, flower-like materials that contain antibodies, enzymes (HRP), and inorganic framework [$Cu_3(PO_4)_2$] were prepared by one-step co-precipitation approach, and were used as APP recognition, signal transduction, and amplification units, in order to enhance the sensitivity of APP detection. In this study, the amount of antibodies and HRP on the property of flower-like materials, the microplate coating procedures, the sensitivity, selectivity, and the utility of this method in human body fluid were systematically investigated, and satisfactory results were acquired. The experimental result also shows that compared with traditional ELISA approach, our method could markedly enhance the sensitivity of the APP detection without complex modification or sophisticated instruments, and is helpful for the screening of intervention of AD.

Experimental

Chemical and materials

The poly-L-lysine (PLL, M.W., 150,000~300,000), bovine serum albumin (BSA), and human serum albumin (HSA) were purchased from Sigma-Aldrich Co. Ltd. (USA), horseradish peroxidase (HRP) was purchased from Adamas reagent Co. Ltd. (Shanghai, China), Tween-20 and glycine were purchased from Dingguo Co. Ltd. (Beijing, China), $CuSO_4$ $5H_2O$ and glucose were purchased from Sinopharm Chemical Reagent, Co. Ltd. (Shanghai, China). Peptides (DAEFR HDSGY EVHHQ K ($A\beta_{1\text{-}16}$), DAEFR HDSGY EVHHQ KLVFF AEDVG SNKGA IIGLM VGGVV IA ($A\beta_{1\text{-}42}$)), random sequence DNA (5'-GGTTA TGGGT GTGAG GAGCG GTGAG TGTAG TGTGA GTGGC G-3') were purchased from Sangon Biotechnology Co. Ltd. (Shanghai, China). 3,3',5,5'-Tetramethylbenzidine (TMB) substrate solutions was acquired from Beyotime Co. Ltd. (Haimen, China). Synthesized amyloid precursor protein (bs-0112P, the sequence is AKERL EAKHR ERMSQ VM, represents the amino acid sequences of APP between the position of 319 to 335), rabbit polyclonal antibody (pAb) specific to amyloid precursor protein (bs-0112R), and HRP conjugated bs-0112R (bs-0112R-HRP) were obtained from Bioss. Co. Ltd. (Beijing, China). All other chemicals were of analytical grade and used without further purification. Double-distilled water (dd H_2O) was used throughout the experiment.

Apparatus

The UV-visible (UV-vis) absorption spectra were recorded on a Tecan Infinite 200 PRO microplate reader (Mannedorf, Switzerland). The Fourier transform infrared (FT-IR) spectra (4000−500 cm^{-1}) were recorded with a Nicolet IS10 FT-IR spectrometer (ThermoFisher Co. Ltd., USA). The X-ray diffraction (XRD) patterns were obtained using an X-ray diffractometer (Bruker, Karlsruhe, Germany) using Cu-Kα radiation (λ=1.5178 Å, 40 kV × 40 mA). The 2θ scanning range was from 5° to 75° with a scanning speed of 0.1° s^{-1}. The pH values of solutions were adjusted using a Leici PHS-3E pH meter (INESA Scientific Instrument Co. Ltd., Shanghai, China). All measurements were carried out at room temperature otherwise mentioned specially.

Scanning electron microscope (SEM) measurement

Scanning electron microscope (SEM) micrographs and energy dispersive X-ray pattern (EDX) of all samples were obtained by a Hitachi S-4800II scanning electron microscope (Hitachi, Japan) operated at an accelerating voltage of 20 kV. The suspensions of flower-like materials were added onto aluminum foil and dried at room temperature, then sputter-coated with gold and imaged by SEM to study the morphologies and elemental compositions of the sample.

Preparation of antibody-HRP-$Cu_3(PO_4)_2$ flower-like materials

The flower-like materials were synthesized according to the one-step co-precipitation method [23, 24]. Generally, 20 μL of aqueous $CuSO_4$ solution (120 mM) was added to 3 mL of PBS (10 mM NaH_2PO_4/Na_2HPO_4, 138 mM NaCl, 2.7 mM

KCl, pH 7.4) containing 0.1 mg/mL HRP and 0.01 mg/mL antibody (bs-0112R), followed by incubation at room temperature for 72 h. The precipitates were collected after centrifugation and dried under vacuum.

The sensing protocols

The sensing protocol is mainly based on the previous reports with some modifications [30, 31]. Briefly, the 96-well plates were coated with different concentrations of PLL for 24 h, then the solutions were discarded, and the wells were washed with PBS-T (10 mM NaH_2PO_4/Na_2HPO_4, 138 mM NaCl, 2.7 mM KCl, 0.05% (v/v) Tween-20, pH 7.4) once, and 50 μL of 1% (v/v) glutaraldehyde in PBS was added to activate the amino groups for 0.5 h (CAUTION: steps with glutaraldehyde should be performed in a fume hood. Disposing of glutaraldehyde waste must be in accordance with local safety and waste management guidelines. This solution should be prepared fresh each day of use) [35, 36], then the PBS-T washing step is repeated and different amounts of APP are added and reacted for 12 h. Afterwards, 1 M glycine is added to block the aldehyde sites for 1 h. After washing with PBS-T, BSA solution is added into each well to decrease the nonspecific adsorption and incubated at 37 °C for 1 h, then the washing step using PBS-T is repeated. The mixtures of as-prepared flower-like materials and different concentrations of APP are added to the wells and incubated at 37 °C for different times. Then the mixtures are removed and the wells are washed with PBS-T three times. Afterwards, 200 μL TMB substrate solutions are added into each well and reacted for different times. Finally, 50 μL H_2SO_4 (2 M) solutions are added into the wells to terminate the chromogenic reaction [37, 38]., and the absorbance spectra of solutions in each well are recorded from 350 nm to 550 nm, respectively. For traditional ELISA experiment, the procedure is the same as the above-mentioned experiment except that 5 μg/mL HRP conjugated antibody specific to APP (bs-0112R-HRP) is used as the substitute of composite flower-like materials.

Real sample preparation and analysis

The human serum samples from healthy people were obtained from Affiliated Hospital of Jiangsu University, and centrifuged at 13,000 rpm for 30 min to remove the potential aggregates [17]; then the supernatant was carefully pipetted out, diluted 10 times using PBS, and applied for the immunoassay.

Results and discussions

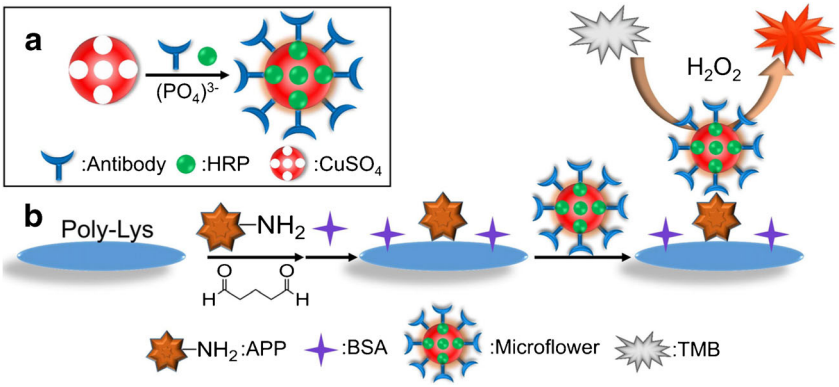
The APP sensing protocol

As shown in Scheme 1, we have developed a novel method for sensitively detecting APP using PLL and APP antibody-HRP-$Cu_3(PO_4)_2$ composite flower-like materials. PLL was used as capture assistant reagent, as it contained numerous $-NH_2$ groups and is always used for pretreating microplates to increase the adhesion of cells or other small molecules [31, 34, 39]. By using glutaraldehyde as crosslinking reagent, the $-NH_2$ groups containing APP can be firmly attached onto microplate [30–34]. On the other hand, the flower-like materials can be easily prepared through one-step co-precipitation approach [21–24, 29], and as there are numerous HRP molecules on one such flower-like material, it is easy to speculate that the detecting signals originated from the recognition of APP by antibody may increase dramatically. By using the competitive immunoassay, we may sensitively and selectively detect APP.

The characterization of flower-like materials

The structure and morphology of flower-like materials have great influence on the APP detection. Therefore, HRP concentration was first investigated to optimize the synthesis conditions. As shown in Fig. 1A–C and Fig. 2, large irregular shaped aggregates, but not flower-like structures, were observed in the absence of HRP. The flower-like structures appeared and the petals increased with increasing the HRP concentrations from 0.01 mg/mL to 0.1 mg/mL. This phenomenon may be the reason that at low concentration of HRP, mainly crystals of $Cu_3(PO_4)_2$ rather than composite species were formed, whereas the bundle-like structure may result from the aggregated HRP proteins when the HRP concentration is too high. According to a previous report [24], during the composite materials formation, the Cu^{2+} first coordinate with the proteins (especially the HRP), and form the scaffold of the composite materials; next, the hierarchical structure are gently enlarged onto the protein-Cu^{2+} scaffold. However, if the proteins are not sufficient (e.g., HRP is absent) as there are not enough nucleation centers, large irregular-shaped $Cu_3(PO_4)_2$ aggregates rather than composite materials may form consequently. On the other hand, the molecule weight of HRP and antibody is about 40 kDa and 150 kDa, respectively, and the low space hindrance of HRP (small size) may favor the quick formation of numerous nucleation centers and thereby facilitate the formation of final hierarchical structures. As the $Cu_3(PO_4)_2$ aggregates have smaller surface area and little internal structure, they cannot incorporate enough functional antibodies and enzymes; therefore, they cannot serve as ideal platforms for biosensing [24].

However, when the concentrations of HRP further increased above 0.1 mg/mL, some bundles appeared on the flower-like materials, accompanied by the formation of some irregular debris in the solution (see Fig. 2 E and J). These debris and bundles have low surface area and are without multi-layered structure, which may hinder the loading of numerous antibodies and HRP on one flower-like composite. The antibodies were used to recognize antigen, and HRP was used to produce measurable signals in the ensuing



Scheme 1 Schematic representation of the preparation of composite flower-like materials **(A)** and the flower-like materials based competitive ELISA for APP detection **(B)**. The illustration is not drawn to scale

chromogenic reaction. The relative amounts of antibody on one flower-like structure may decrease by increasing the concentrations of HRP in the initial solutions, which will not further increase the signal intensity or even limit the interaction of antibody and antigen. We may therefore conclude that suitable concentrations of antibodies and HRP are crucial for the formation of flower-like material structure. The detailed forming process of flower-like structures should be studied further in the next experiment.

Different characterization methods, including scanning electron microscopy (SEM), Fourier transform infrared (FT-IR) measurement, energy dispersive X-ray pattern (EDX), and X-ray diffraction (XRD) measurement were employed to further characterize the composite flower-like materials prepared using 0.1 mg/mL HRP. As shown in Fig. 1A, blue unconsolidated precipitates were acquired, which is the same as the color of $Cu_3(PO_4)_2$; SEM images exhibited hierarchical peony-like flower morphology, which was formed by hundreds of nanoplates (see Fig. 1B and C). This experimental result is consistent with the previous reports that the flower-like materials were formed through proteins as nucleation center, and $Cu_3(PO_4)_2$ as glue to assemble the proteins and formed complicated structures, which possess a large ratio of surface to volume [22, 24]. The FT-IR spectra suggest the formation of composite flower-like materials by the interaction of HRP and $Cu_3(PO_4)_2$; as shown in Fig. 1D, the strong IR bands of $Cu_3(PO_4)_2$ at 1054 and 1150 cm^{-1} were attributed to P−O and P=O vibrations, whereas the IR band of HRP at 1400−1600 cm^{-1} was attributed to −CONH, and 2800−3000 cm^{-1} was attributed to $-CH_2$ and $-CH_3$. Moreover, the IR

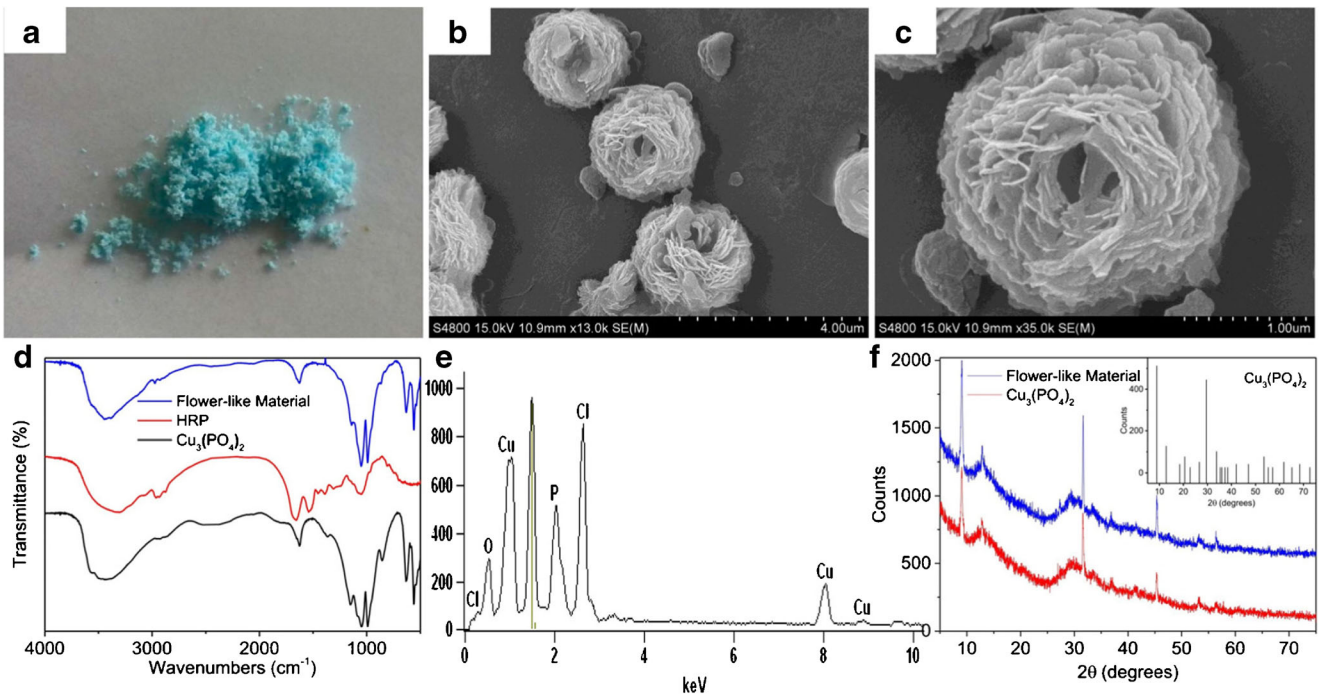


Fig. 1 Photograph **(A)**, SEM images (low magnification **B**, and high magnification **C**), FT-IR spectra **(D)**, EDX patterns **(E)**, and XRD patterns **(F)** of the composite flower-like materials, respectively. The concentrations of $CuSO_4$, HRP, and antibody used for synthesizing flower-like materials are 0.8 mM, 0.1 mg/mL, and 0.01 mg/mL, respectively

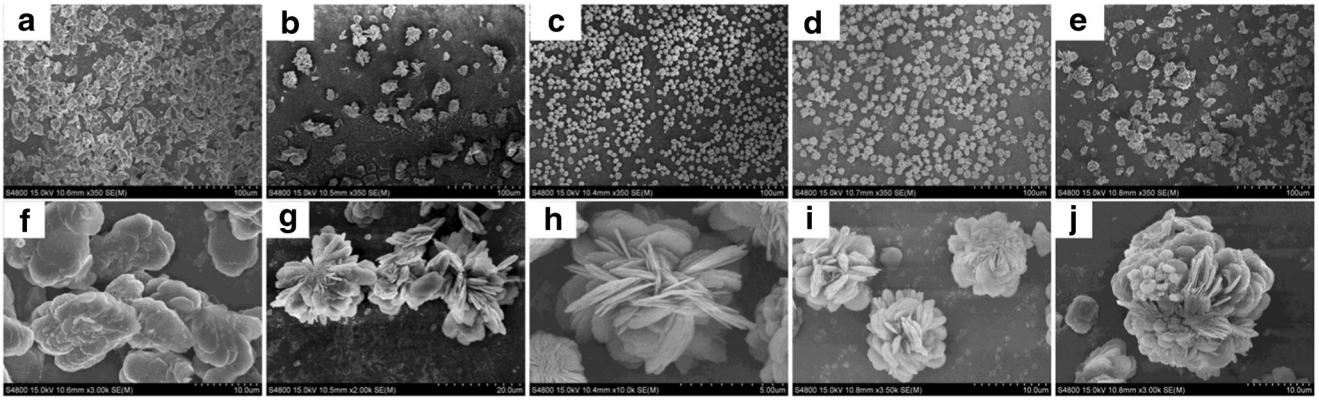


Fig. 2 The SEM images (low magnification **A**–**E**, and high magnification **F**–**J**) of the composite flower-like materials synthesized using 0 (**A** and **F**), 0.01 (**B** and **G**), 0.02 (**C** and **H**), 0.05 (**D** and **I**), and 0.5 (**E** and **J**) mg/mL HRP, respectively. The concentrations of $CuSO_4$ and antibody used for synthesizing flower-like materials are 0.8 mM and 0.01 mg/mL, respectively

spectra of composite flower-like material did not show new absorption peaks or significant peak shift in comparison with HRP, which indicates that HRP was immobilized via self-assembly instead of covalent bonding [23]. The EDX experimental result indicates that the formed flower-like material was composed of Cu, P and O, the unlabeled peak indicates the aluminum foil substrate (see Fig. 1E); the sharp, strong peaks in XRD pattern further confirmed that the structure of composite flower-like material is well crystallized and is the same as $Cu_3(PO_4)_2$, and the positions and relative intensities of all diffraction peaks of flower-like material matched well with those peaks shown in JCPDS card (00-022-0548, $Cu_3(PO_4)_2$) (see Fig. 1F). These experimental results indicate that hierarchical $Cu_3(PO_4)_2$ flower-like materials contained numerous HRP and antibodies were prepared successfully.

Optimization of the detecting conditions

A variety of factors can affect the performance of this flower-like material-based assay. As shown in Fig. 3A, the absorbance at 450 nm of the detection system (A_{450nm}) was increased by increasing the concentration of PLL from 0 to 200 μg/mL, which indicated that PLL is able to increase the microplate coating efficiency. The impact of immobilized APP on the performance of detection was also studied, as more APP attached onto the wells may combine more flower-like materials, and result in more chromogenic signals. By increasing the concentrations of immobilized APP from 0 to 2 μg/mL, the A_{450nm} also increased consequently (see Fig. 3B). Bovine serum albumin (BSA) as a blocking reagent, its concentration was also important for the performance of this immunoassay, by increasing the concentration of BSA from 0 to 2 % (w/v), the A_{450nm} decreased consequently (see Fig. 3C). This may be because BSA can decrease the non-specific adsorption on the microplate, whereas high concentration of BSA may interfere with the interaction of immobilized APP and antibody functionalized flower-like materials [22]. For HRP-containing composite materials, the amount of flower-like material was crucial to the APP detection; as shown in Fig. 3D, the A_{450nm} increased by increasing the concentration of flower-like materials from 0 to 200 μg/mL. This confirmed that the more flower-like materials used, the more HRP and antibodies were attached onto the wells of the microplate. The performance of flower-like materials synthesized using different concentrations of HRP was also investigated. By increasing the concentrations of HRP from 0 to 0.5 mg/mL, the A_{450nm} increased (see Fig. 3E). This may be because more HRP molecules were adsorbed onto the flower-like materials when higher concentration of HRP was used, and resulted in the detection of enhanced signal. The reaction time between immobilized APP and flower-like materials was also investigated. The A_{450nm} increased by increasing the reaction time from 0 to 90 min (see Fig. 3F). In order to acquire the optimal detection conditions, the differences of A_{450nm} of the solutions in the absence and presence of 1 ng/mL APP were investigated, at 100 μg/mL PLL, 0.5 μg/mL immobilized APP, 0.5 % BSA, 50 μg/mL flower-like material, 0.1 mg/mL HRP, and 50 min reaction time between immobilized APP and flower-like materials. The changes of A_{450nm} (ΔA_{450nm}) were most significant; therefore, these experimental parameters were used for detecting APP afterwards.

Detection of APP based on flower-like materials

By using the selected optimal conditions, the composite flower-like material-based ELISA system was used to quantitatively determine the concentration of APP. As shown in Fig. 4A and B, the absorbance of the detecting system gradually decreased with the concentration of APP increasing, consequently. The A_{450nm} also decreased with increasing the concentration of APP from 0.1 to 100 ng/mL [the calibration curve is A_{450nm}= 0.884-0.359 $\log C_{APP}$ (ng/mL)], the limit of detection (LOD) is 0.3 ng/mL (three times of standard deviation of the blank, 3σ). Inset of Fig. 3B shows the representative images of the mixtures; the color of the solution changed

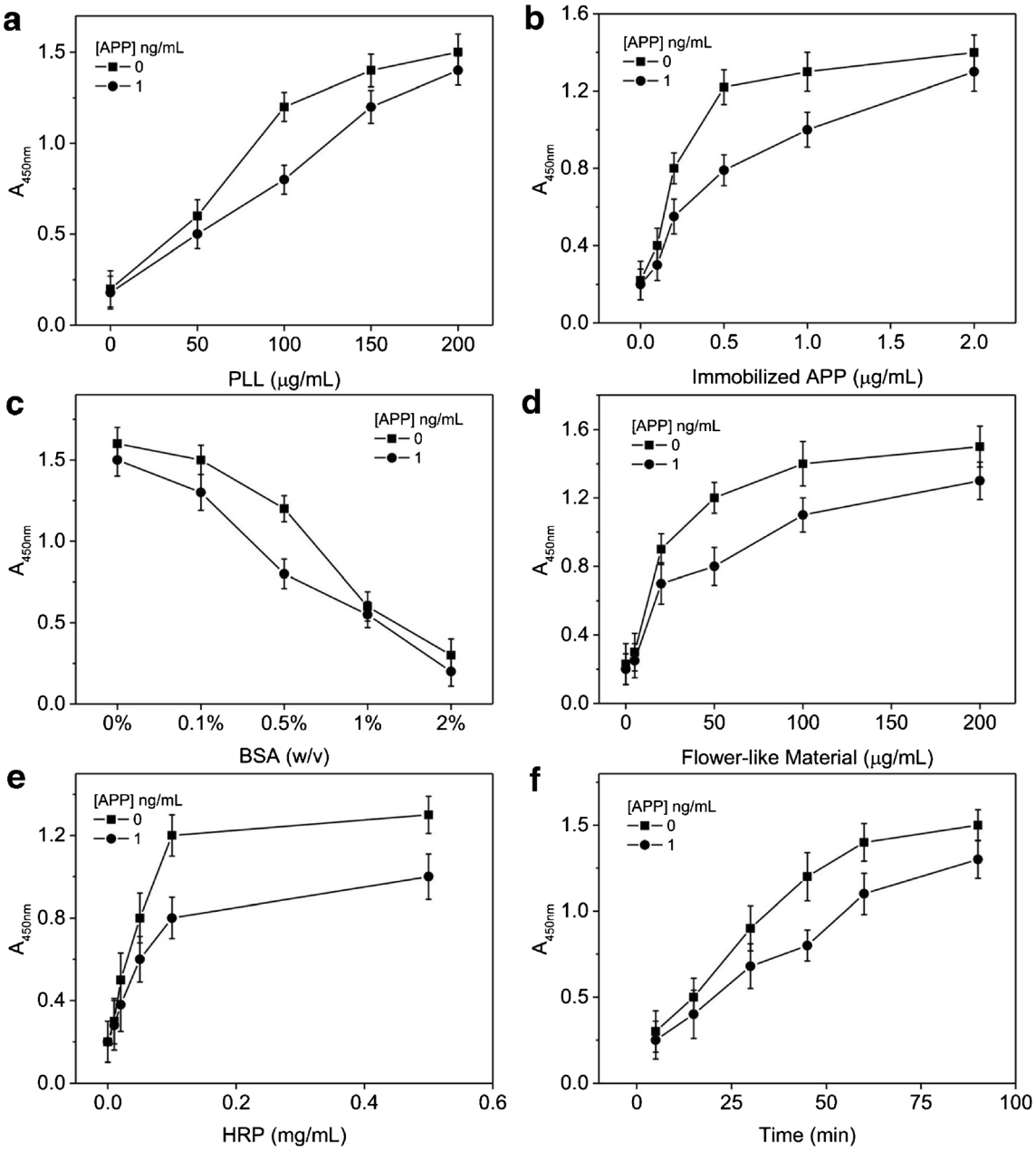


Fig. 3 The A_{450nm} changes of the detecting system in the absence and presence of 1 ng/ mL APP with the addition of different concentrations of PLL **(A)**, immobilized APP **(B)**, BSA **(C)**, flower-like materials **(D)**, flower-like materials synthesized by different concentrations of HRP **(E)**, and as a function of the interaction time between immobilized APP and flower-like materials, respectively. The error bars represent the relative standard deviation of three experimental results

from deep yellow to pale-yellow, and finally approaching colorless when the concentration of APP increased successively. Therefore, it is possible to estimate the concentrations of APP in the solution by comparing the color of the product with the standard solutions containing certain amounts of APP. which is probably used for assisting semi-quantitative determination of APP by naked eyes.

To assess the specificity of this assay, interferences, including the Aβ analogues ($A\beta_{1\text{-}16}$ and $A\beta_{1\text{-}42}$), human serum albumin (HSA), glucose, and random DNA were detected by our proposed method, as shown in Fig. 4C. Only APP can dramatically decrease the A_{450nm} of the detection system, which suggests that this assay is specific to APP. We also evaluated the stability of flower-like material, the material shows acceptable stability in 30 d (see Fig. 4D).

To compare with traditional single antibody-HRP-based assay, HRP conjugated APP antibody (bs-0112R-HRP) was used as the substitute of the composite flower-like materials; PLL was first coated on the microplate, then APP was conjugated with PLL (without PLL coating, the amount of APP adsorbed

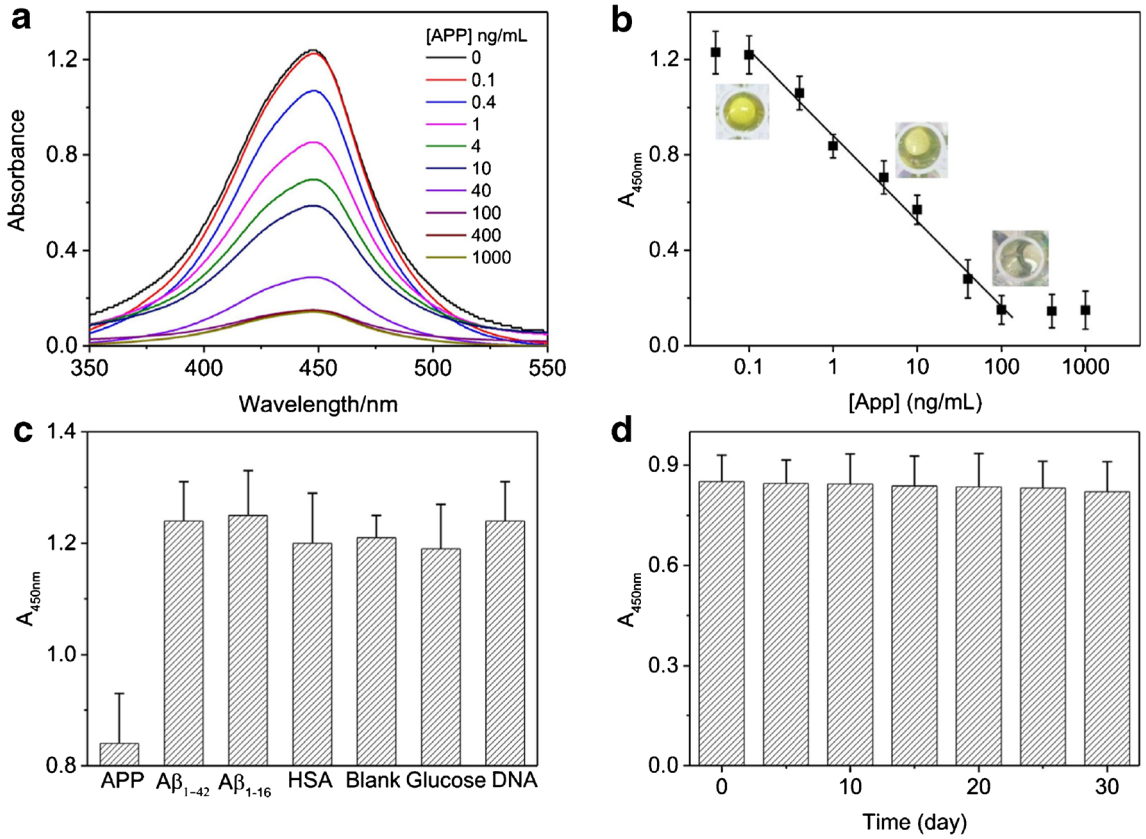


Fig. 4 The UV-visible spectral changes of composite flower-like materials based detecting system in the presence of different amounts of APP **(A)** and the changes of A_{450nm} as a function of the concentrations of APP **(B)**. Inset of **(B)** indicates the images of microplate wells in the presence of 0.1, 4, and 100 ng/mL of APP, respectively. The A_{450nm} of the detecting system in the presence of 1 ng/mL different compounds **(C)**, and the A_{450nm} of the detecting system using flower-like materials stored for a different time, respectively. The error bars represent the relative standard deviation of three experimental results. The concentrations of $CuSO_4$, HRP, and antibody used for synthesizing flower-like materials are 0.8 mM, 0.1 mg/mL, and 0.01 mg/mL, respectively. Other concentrations of compounds or experimental conditions are 100 μg/mL PLL, 0.5 μg/mL immobilized APP, 0.5 % BSA, 50 μg/mL flower-like material, and 50 min reaction time between immobilized APP and flower-like materials

on the microplate is negligible, as there is no difference of detection signal between APP samples and buffer alone, data not shown here), after BSA was added to decrease the unspecific interaction, solutions contained APP and bs-0112R-HRP were added, then TMB and H_2O_2 were added, and finally the absorbance of the solutions was recorded after the addition of H_2SO_4. As shown in Electronic Supplementary Material (ESM) Fig. S1A, the signal intensity of our flower-like material-based method enhanced significantly compared with traditional methods. Meanwhile, compared with our method, the traditional approach had relatively low detection performance, as the limit of detection was about 10 times higher than our method (3 ng/mL and 0.3 ng/mL, respectively), and the linear detection range was also relatively narrow (1–100 ng/mL and 0.1–100 ng/mL, respectively) (see ESM Fig. S1B). This may originate from the binding efficiency of small molecules onto the microplate being relatively low without the poly-L-lysine as the anchoring molecules; meanwhile, the relatively high loading capacity of HRP and antibody on composite materials may also result in the difference of signal intensity between our method and traditional ELISA approach without using the antibody-HRP-$Cu_3(PO_4)_2$ composite materials. Although both methods can be used to detect APP, the preparation of function materials is simpler, and the detection of APP is cost-efficient compared with antibody-HRP conjugates.

It is significant to detect APP in real body fluid samples for the detection and cure of AD. As shown in Table 1, different amounts of APP were added into human serum and detected using the above-mentioned method; the recovery was between

Table 1 Determination of APP concentrations in human serum samples ($n=3$)

Human serum (No.)	Added (ng/mL)	Detected (ng/mL)	Recovery (%)	RSD (%)
1	0.5	0.47±0.04	94	8.5
2	1	1.05±0.08	105	7.6
3	10	10.8±0.9	108	8.3
4	40	42±1.8	105	4.3

94% and 108% with the relevant standard deviation (RSD) below 9%. We also selected serum samples of three persons, and detected the APP concentration using our proposed method utilizing antibody-HRP-$Cu_3(PO_4)_2$ composite materials and the traditional ELISA method utilizing enzyme linked antibody. As shown in ESM Table S1, the detected amounts of APP using our method are similar to the result obtained from ELISA method using enzyme linked antibody, which indicated that our method has reasonable accuracy. It is noteworthy that if directly detecting these serum samples without the addition of APP, as the enzyme linked antibody based ELISA method has lower sensitivity, the APP is not detectable in these samples, since the concentration is below the limit of detection. Our method can be used for determining the APP in two serum samples (although in one sample, the APP is also below the limit of detection). These results suggest that our developed flower-like material-based ELISA can be used for determining APP concentration in clinical samples.

In the next experiment, we may try to obtain more clinical samples (e.g., urine, cerebrospinal fluid) from different people (e.g., different age, different gender, and different neurodegenerative disease, et al.) and to explore further applications of our method. This work is in progress in our lab.

Conclusions

A flower-like composite material was used to detect APP; PLL was used for coating the surface of microplate wells which favor the immobilization of APP on the plates. Antibody-HRP-$Cu_3(PO_4)_2$ composite flower-like materials were used to detect APP, the property of composite materials did not change a lot in 1 mo. Although APP was chosen here to establish this assay, we speculate that the method could be modified by changing the antibodies in the future, and possible to be used to detect other species, such as Aβ in AD, prion in Huntington's disease, and α-synuclein in Parkinson's disease, which may be meaningful in the near future.

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Compliance with ethical standards

This is not a clinical study on humans with an ethics committee. The biological samples were transferred to our laboratory from the Affiliated Hospital of Jiangsu University. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of interest

The authors declare that they have no competing interests

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