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A paper-supported aptasensor for total IgE based on luminescence resonance energy transfer from upconversion nanoparticles to carbon nanoparticles

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ABSTRACT

A paper-supported aptasensor was constructed for total IgE using a luminescence resonance energy transfer (LRET) protocol with upconversion nanoparticles (UCNPs) as energy donors and carbon nanoparticles (CNPs) as energy acceptors. This is the first time that zero-dimensional carbon nanoparticles were used as energy acceptors for paper-based LRET assays. The π-π stacking interaction between the aptamer and CNPs brought the energy donor (UCNPs) and energy acceptor (CNPs) in close proximity, induced the LRET process on the surface of paper and thus led to the luminescence quenching of UCNPs. The introduction of IgE inhibited the energy transfer and hence recovered the luminescence of UCNPs in a concentration-dependent manner, as a result of the recognition between IgE and aptamer. This aptasensor can be used to detect IgE concentration in the range of 0.5–80 ng/mL in both buffer solution and human serum samples. The IgE concentrations measured by our method were well correlated to those obtained from chemiluminescence-based clinical assay. Owing to its simplicity and accuracy, the proposed sensor thus showed the potential of clinical applications.

1. Introduction

Paper-based assay hold great promise to practical cost-effective applications, such as point-of-care testing and consumer diagnostics, especially in under-developed regions lacking the laboratory resources. This is particularly owing to the apparent advantages of paper substrate such as low cost, three-dimensional fibrous structures and large surface area, easy patterning and chemical modification, and the capillary wicking action for fluid flow [1–3]. Up to now, paper-based sensors have been used in medical diagnosis, environmental monitoring, and food quality control and so on [4–6]. In recent years, an increasing number of researchers have sought to integrate nanomaterials with paper-based assay formats. The rapidly developing nanotechnology can improve the quality of the paper-based devices due to the unique properties of nanomaterials. So far, AuNPs [7], magnetic nanoparticles [8], ceria nanoparticles [9], quantum dots (QDs) [10], UCNPs [11] and carbon materials [12–16] etc. have been employed in paper-based sensors and used as labels, carriers or other functions such as surface enhanced raman scattering (SERS), surface plasmon resonance (SPR) [17,18].

UCNPs are promising luminescent materials for the construction of biosensor in body fluids owing to their features of excitation with near-infrared (NIR) light and anti-Stokes emission, which can circumvent the problem of autofluorescence and/or light scattering [19–21]. In consideration of the high complexity of both clinical samples and paper substrates, UCNPs would be particularly suitable for paper-based clinical analytics. To date, UCNPs have been successfully used as fluorescence signal reporter or energy donor in luminescence resonance energy transfer (LRET) system in some literatures [22–25]. Nonetheless, the research on paper-based analytical device based on UCNPs (UC-PADs) is just on its initial stage and needs further improvement in many aspects, such as the flexibility and diversity of assay model, the accuracy and robustness in clinical samples. On the other hand, carbon materials, such as graphite, carbon nanotubes (CNTs), carbon nanofibers (CNFs), and graphene oxide (GO) have been verified as effective energy accep-

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tors in solid assays [26]. There are also reports using GO as the energy acceptor to detect DNA, protein and pathogen on the surface of paper [27–29]. However, no report using CNPs as energy acceptor in solid matrix has been made known to public.

In this work, we report a paper-supported aptamer biosensor for IgE detection using UCNPs and CNPs as the energy donor-acceptor pair. To the best of our knowledge, this is the first report using CNPs in the solid matrix as the acceptor for energy transfer. We chose immunoglobulin E (IgE), which plays a major role in allergic diseases [30], as the model analyte and the D17.4 IgE aptamer as recognition unit. This aptasensor not only reduces the background signal due to the "off-on" switching model during the sensing but also avoids the pretreatment of samples (owing to the unique merit of UCNPs), providing high sensitivity and specificity.

2. Materials and method

2.1. Chemicals and reagents

Immunoglobulin E was purchased from Shanghai Linc-Bio Science Co., Ltd. (Shanghai, China). Polyacrylic acid (PAA, with an average molecular weight of 1800), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl), N-hydroxysulfosuccinimide sodium salt (Sulfo-NHS), 2-(N-morpholino) ethanesulfonic acid (MES), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and tris (hydroxymethyl) aminomethane (Tris) were from Sigma-Aldrich. Human IgG, lysozyme and bovine serum albumin (BSA) were from Zhongshan Golden Bridge Biotechnology Co., Ltd. (Beijing, China). The rest of the chemical reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and at least of analytical grade. All aqueous solutions were prepared using ultrapure water (Mill-Q, Millipore, 18.2 MΩ resistivity). Amine modified IgE aptamer (5′-NH_2-AAAAAGGGGCACGTTTATCCGTCCCTCCTAGTGGCGTGCCCC-3′), with five A bases next to the amine group as the spacer and the rest bases as the IgE aptamer, was supplied by Sangon Biotechnology Co., Ltd (Shanghai, China).

2.2. Instrumentation

The size and morphology of UCNPs and CNPs on the surface of paper were characterized by Zeiss SIGMA FESEM (Carl Zeiss, Germany). The crystal phase of UCNPs was identified by X'Pert Pro X-Ray Diffractometer (XRD) (PANalytical, Holland) with 2θ range from 10° to 80° at a scanning rate of 4° per minute, with Cu Kα irradiation (k = 1.5406 Å). UV–vis absorption spectra data were recorded with an UV-2550 spectrophotometer (Shimadzu, Tokyo, Japan). Fluorescence spectra were collected with a RF-5301PC fluorescence spectrophotometer (Shimadzu, Tokyo, Japan) with an external 980 nm CW laser (Beijing Hi-Tech Optoelectronic Co., Ltd.).

2.3. Synthesis of upconversion nanoparticles

$NaYF_4$: Yb, Er upconversion nanoparticles were synthesized according to the previously reported method [20,21]. Firstly, oleic acid-stabilized UCNPs were synthesized by a solvothermal method. Rare-earth stearate $Ln(oleate)_3$ (Y/Yb/Er = 0.80: 0.18: 0.02, mole-to-mole ratio) was used as the precursor for the synthesis of oleic acid-stabilized UCNPs (OA-UCNPs). Briefly, 1 mmol of $Ln(oleate)_3$, 20 mmol of NaF, 10 mL of oleic acid (OA) and 10 mL of 1-octadecene (ODE) were added to a 100 mL three-necked flask simultaneously and the mixed solution reacted at 110 °C with magnetic stirring for 1 h under an argon flow to obtain a transparent yellow solution, followed by heating to 290 °C and maintaining at this temperature for 2 h under argon atmosphere. After cooling down to room temperature, the resulting nanoparticles were precipitated out by the addition of equal volume of ethanol, collected by centrifugation, and washed several times with ethanol and cyclohexane (v/v, 4:1). Finally, the synthesized OA-$NaYF_4$: Yb, Er nanoparticles were redispersed in chloroform before further treatment.

A ligand exchange strategy was used to obtain PAA-UCNPs. Briefly, 30 mL diethylene glycol (DEG) and 600 mg PAA were added to a 100 mL three-necked flask simultaneously and the mixed solution was vacuumed and heated to 110 °C. 8 mL of OA-UCNPs solution was added and the mix solution reacted at 110 °C for 1 h, followed by heating to 290 °C and maintaining at this temperature for 6 h under argon atmosphere. After cooling down to room temperature, the resulting nanoparticles were precipitated out by the addition of equal volume of ethanol, collected by centrifugation, and washed several times with ethanol and water. The product was dried under vacuum before use.

2.4. Synthesis of carbon nanoparticles

Carbon nanoparticles (CNPs) were synthesized with candle soot as starting material according to the reported method with some modifications [31]. Briefly, 18 mg of candle soot was added to the mixture solution containing 9 mL of HNO_3 and 9 mL of N, N-Dimethylformamide (DMF), and then the solution was refluxed and stirred at 100 °C for 18 h. After the mixture was cooled down to room temperature, a precipitate was obtained by centrifuging and washed with water for three times. Then the product was dried under vacuum and redispersed in water before use. The concentration of CNPs was calculated as 0.5 mg/mL.

2.5. Attachment of the IgE aptamer to UCNPs

The amino modified IgE aptamer was covalently conjugated to PAA-UCNPs using EDC·HCl and Sulfo-NHS as the cross-linking agents according to previous works [32]. Briefly, 1 mg of PAA-UCNPs was dissolved in 1 mL of MES buffer solution (10 mM, pH 5.5). Then 0.6 mg of EDC·HCl and 1.2 mg of Sulfo-NHS were added to the solution and the mixture solution was incubated at room temperature with gentle shaking for 40 min to activate the carboxyl groups of PAA-UCNPs. The activated PAA-UCNPs were collected after washing with water for three times. The obtained precipitate was redispersed in 1 mL of HEPES buffer solution (10 mM, pH 7.2) containing 1 nmol of IgE aptamer. Then the mixture was maintained overnight at room temperature with gentle shaking and 10 mg of Tris was added to the mixture to block the excess NHS. The as-prepared UCNPs-IgE aptamer conjugate was harvested by centrifugation and washed with ultrapure water for three times. Finally, the product was re-dispersed in 1 mL of PB buffer solution (10 mM, pH 7.4) and stored at 4 °C for further use. The concentration of UCNPs-IgE aptamer was calculated as 1 mg/mL.

2.6. Construction of paper-supported upconversion fluorescence detection devices

The permanent markers were used to directly plot paper-supported analytical devices with the aid of plastic templates with specific pattern [33,34]. We plotted the pattern on the whatman no. 1 paper according to template with a permanent marker and left the resultant patterned paper at room temperature to evaporate the solvent. The resins remaining in the marks in paper would form the hydrophobic barriers to separate the independent test zones. The upconversion luminescence signals were measured by self-made device. Briefly, the paper loaded with reagents and samples in the test zones was stuck on the surface of solid sample holder and placed in quartz cuvette diagonally followed by recording the