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Nanoparticle-mediated photothermal effect enables a new method for quantitative biochemical analysis using a thermometer

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1. Experimental Section

1.1. Materials and Instruments

Carboxyl-functionalized iron oxide nanoparticles (NPs) with diameters of 40 nm were purchased from Ocean NanoTech LLC (USA). Polyclonal rabbit anti-human PSA antibody, monoclonal mouse anti-human PSA antibody and carcino-embryonic antigen (CEA) were purchased from Abcam (USA). Prostate-specific antigen (PSA), bovine serum albumin (BSA) and serum from normal human male AB plasma were obtained from Sigma-Aldrich (USA). Hepatitis B surface antigen (HBsAg) was acquired from Fitzgerald Industries International Inc. (USA). Prussian blue (PB) NPs were typically prepared according to the literature (*Chem. Commun., 2012, 48, 11567–11569*). Otherwise stated, all other chemicals were of analytical grade and used as received.

The diode laser with the wavelength of 808 nm and the output power intensity adjustable from 0 to 2.5 W was obtained from Opto Engine LLC (USA). The KT-300 LCD pen-style digital thermometer with the detection range from -50 to +300 ^{o}C was purchased from a local supermarket. Photographs were taken with a Canon EOS 600D camera.

1.2. Preparation of Antibody-conjugated Iron Oxide NPs

Polyclonal rabbit anti-human PSA antibody was covalently conjugated to carboxyl-functionalized iron oxide NPs through the typical carbodiimide method. Typically, 1.0 mg iron oxide NPs were dispersed in 2.0 mL deionized water with ultrasonication. The mixture (25.0 μL) of N-hydroxysulfosuccinimide (Sulfo-NHS) and 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) aqueous solutions with the same concentration of 25 mg·mL^{-1} was added to the nanoparticle dispersion, followed by reaction at room temperature for 30 min under gentle stirring. 80.0 μg polyclonal rabbit anti-human PSA antibody was then added into the above

nanoparticle dispersion, followed by reaction at room temperature for 2 h under gentle stirring. The nanoparticle dispersion was centrifuged at 11,000 rpm for 10 minutes at 4 ^{o}C to collect the antibody-conjugated iron oxide NPs, which were then washed with PBS (pH=7.4, 0.01 M) for 3 times. The antibody-conjugated iron oxide NPs were finally dispersed in 2.0 mL PBS (pH=7.4, 0.01 M) containing 0.2% BSA. The nanoparticle dispersions were stored at 4 °C before use.

1.3. Sandwich-type Immunoassay

A 100 μL monoclonal mouse anti-human PSA antibody solution with a concentration of 30 μg·mL^{-1} was added in each PCR tube (200 μL) and incubated for 12 h at 4 ^{o}C. 200 μL blocking buffer containing 5.0% BSA was then used to block the tubes for 2.0 h at 37.5 ^{o}C, followed by incubation with different concentrations of standard PSA solutions containing 5.0% BSA for 2.0 h at 37.5 ^{o}C. After thoroughly washing, 100 μL polyclonal anti-PSA antibody-conjugated iron oxide NPs suspensions (0.5 mg·mL^{-1}) were added for further incubation at 37.5 ^{o}C for 2.0 h. Finally, the PCR tubes were thoroughly washed with PBS.

To transform iron oxide NPs captured in the sandwich immunoassay system into Prussian blue (PB) NPs, 120 μL HCl solutions (0.1 M) were added into the tubes, followed by ultrasonication for 60 min at room temperature. 30 μL potassium ferrocyanide aqueous solutions (90 mM) were then added into the tubes to produce PB NPs from the reaction between ferric ions and ferrocyanide ions under acidic conditions. The immunoassay solutions were thoroughly mixed every 10 min, which were finally used for the photothermal measurement, UV-Vis spectroscopic characterization, Fourier transform infrared spectroscopic (FTIR) and Transmission electron microscopic (TEM) characterization after reaction for 1.0 h.

1.4. Characterization

UV-Vis spectroscopy, FTIR and TEM were used to characterize the immunoassay solutions before and after the iron oxide-to-PB NPs transformation process. UV-Vis spectra of the immunoassay solutions was performed on a SPECTROstar Nano Microplate Reader (BMG LABTECH) using a 96-well microplate. FTIR was performed on a Spectrum 100 FT-IR spectrometer (PerkinElmer, Inc.) to characterize the change of the immunoassay solutions before and after the iron oxide-to-PB NPs transformation process. Immunoassay solutions before and after the transformation process at PSA concentration of 32.0 $ng \cdot mL^{-1}$ were dropped on Whatman® cellulose chromatography papers (Sigma-Aldrich), followed by drying at room temperature for FTIR characterization using the chromatography paper as the blank. TEM was carried out to observe the morphology of nanoparticles in the immunoassay solutions using a JOEL 3200FS cryo-Transmission electron microscope. Immunoassay solutions before and after the transformation process at PSA concentration of 32.0 $ng \cdot mL^{-1}$ were deposited on carbon-coated copper grids for TEM characterization.

1.5. Photothermal Detection Protocol

For monitoring of temperature elevation of iron oxide NPs and PB NPs during the irradiation process for 10 min, 1.0 mL of the nanoparticle dispersions in disposable UV cuvettes were irradiated with a 808 nm laser at a power density of 3.12 $W \cdot cm^{-2}$ for 10 min. A pen-type digital thermometer without exposure to the laser was inserted into the nanoparticle dispersions to monitor the temperature. The temperature was recorded every 10 seconds during the irradiation process for 10 min.

For photothermal immunoassay, the PCR tubes containing 0.15 mL immunoassay solutions

were exposed to a 808 nm laser at a power density of 5.26 $W \cdot cm^{-2}$ for 1.5 min. After the irradiation, a pen-style digital thermometer was immediately inserted into the solutions to monitor the temperature.

1.6. Photothermal Detection in Human Serum Samples

Serum samples from normal human were used to validate the reliability of the developed photothermal immunoassay. 10 μL different concentrations of standard PSA solutions were spiked into 1.0 mL 3-fold diluted human serum to prepare the real serum samples with final PSA concentrations of 4.0, 8.0 and 16.0 $ng \cdot mL^{-1}$, respectively. After thoroughly mixing, the concentrations of PSA in the spiked serum samples were tested with the developed photothermal immunoassay to evaluate the recoveries of PSA spiked in the serum samples using unspiked human serum as the blank.

2. Supplementary Result

Fourier transform infrared spectroscopy (FTIR) was performed to characterize the change of nanoparticles in the immunoassay solution at a PSA concentration of 32.0 ng·mL^{-1} before and after the nanoparticle transformation process. An obvious stretching band was observed at 2085 cm^{-1} (**Figure S1a**) in the FTIR spectrum of the immunoassay solution after the nanoparticle transformation process, while no band was observed before the process (**Figure S1b**). The stretching band corresponded well with that of PB NPs (**Figure S1c**) attributed to the CN stretching in the formed [Fe^{II}–CN–Fe^{III}] structure, [1, 2] indicating the presence of PB in the immunoassay solution after the nanoparticle transformation process.

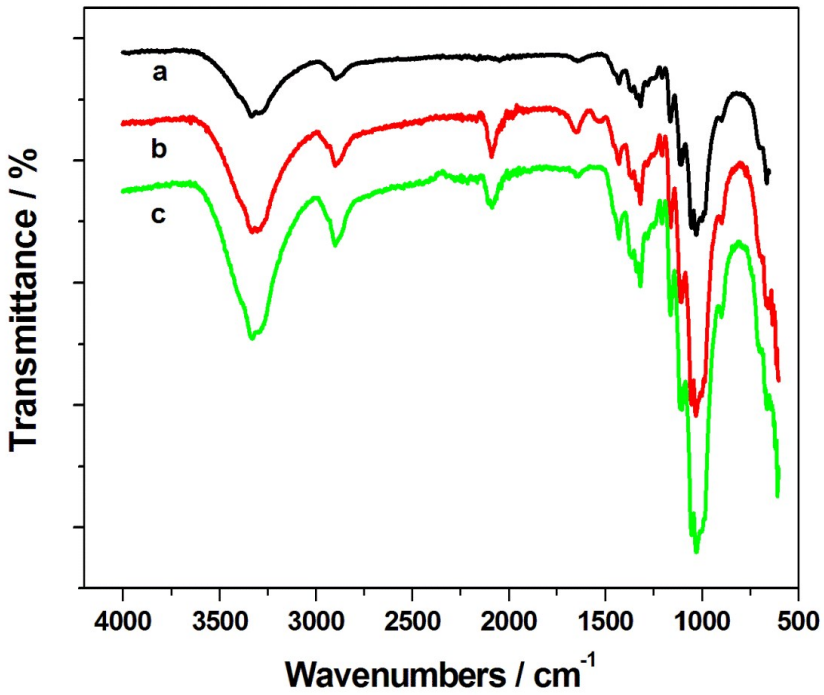

Figure S1. FTIR of the immunoassay solution obtained from 32.0 ng·mL^{-1} PSA. (a) Before and (b) after the reaction with potassium ferrocyanide. (c) FTIR of PB NPs.

Table S1. Photothermal immunoassay of PSA in spiked human serum samples (n=4)

Serum sample Number	Standard PSA concentration ($ng \cdot mL^{-1}$)	Temperature increase (^oC)	Measured PSA concentration ($ng \cdot mL^{-1}$)	Recovery (%)
1	4.0	8.1±0.6	3.67±0.18	91.7±4.4
2	8.0	15.6±0.7	7.70±0.46	96.3±5.8
3	16.0	22.5±0.8	15.30±0.99	95.8±6.2

3. References

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