Supporting Information

Multifunctional mesoporous silica-gold nanocluster hybrid platform for selective breast cancer cell detection using catalytic amplification–based colorimetric assay

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Materials and Methods

Materials and characterizations
Gold(III) chloride trihydrate (HAuCl₄·3H₂O), lysozyme, sodium hydroxide, triethanolamine, triethylamine and cetyltrimethylammonium p-toluenesulfonate (CTAT) were purchased from Sigma-Aldrich. N,N-dimethylformamide (DMF), tetraethoxysilane (TEOS), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl), (3-aminopropyl)triethoxysilane and ethanol were from Alfa Aesar. N-hydroxysulfosuccinimide (Sulfo-NHS) was purchased from Thermo Fisher Scientific. Hydrochloric acid (HCl, 37 %) was obtained from Acros Organics. Human ErbB2/Her2 antibody (anti-HER2) was synthesized by R&D Systems. Breast cancer tissue array (HER2: 3+/2+/1+/0) were obtained from US Biomax. All other chemicals were analytical reagent grade and used as received without further purification. The zeta potential and size distributions were measured with the ZetaPALS zeta potential analyzer. Transmission electron microscopy (TEM) images were obtained with a JEOL 2100 transmission electron microscope. Absorbance measurements were acquired on the spectramax plus 384 spectrophotometer. N₂ adsorption-desorption isotherms were recorded on a Micromeritics ASAP 2020M automated sorption analyzer. The specific surface area and the pore size distributions were evaluated and calculated through the Brunauer-Emmett-Teller (BET) and Barrett-Joyner-Halenda (BJH) methods.

Preparation of lysozyme-protected gold nanoclusters
The lysozyme-protected gold nanoclusters were prepared by previously reported methods with a slight modification. To be specific, the gold nanoclusters were synthesized by chemical reduction of HAuCl₄, with lysozyme as the soft template. Briefly, lysozyme (5 mL, 20 mg/mL) was added to 5 mL of 10 mM HAuCl₄, followed by addition of NaOH (5 μL, 1 M). The mixed solution was stirred for 12 hours in the dark at 37 °C. The product was then stored at 4 °C for further use.

Synthesis of mesoporous silica nanoparticles (MSNs)
The mesoporous silica nanoparticles were synthesized according to a previously reported method with modifications. Typically, CTAT (1.5 g) and triethanolamine (241 µL) were dissolved in 78.0 mL water and stirred for 1 h at 80 °C. After that, TEOS (11.39 g) was added dropwise, and the reaction was performed at 80 °C for another 2 h. Subsequently, the solution was cooled to room temperature while stirring. The product was collected by centrifugation at 10,000 rpm for 10 min, washed with water and ethanol thrice and dried under vacuum. Then, 4.0 g of as-prepared sample was extracted thrice with a solution of 13 mL of HCl (37 %) and 300 mL of ethanol by refluxing at 80 °C for 12 h to efficiently remove the CTAC template. Finally, the nanoparticles were washed with water and ethanol thrice, and dried under vacuum.

Preparation of amino-functionalized MSNs
400 mg of MSNs were first dispersed in 200 mL of ethanol and sonicated for 30 min to break aggregates. After that, 0.4 mL of (3-aminopropyl)triethoxysilane was added, and the mixture was stirred for 0.5 h at room temperature. Then, the reaction solution was refluxed at 80 °C overnight. The product was separated by centrifugation, washed with...
ethanol thrice, and dried under vacuum.

**Synthesis of carboxyl-functionalized MSNs**
The carboxylic acid functionalization of MSN was achieved according to a reported procedure with minor modifications. Briefly, the obtained amino-functionalized MSNs (150 mg) were dispersed in DMF and sonicated for 30 min. Then, succinic anhydride (900 mg) and triethylamine (50 µL) were added, and the reaction was performed at 50 °C for 24 h. After centrifugation and wash with ethanol and water 3 times, the final product was obtained as a white powder after lyophilization.

**MSN-AuNCs-anti-HER2 preparation**
10 mg of carboxyl-functionalized MSNs was suspended in 5 mL of PBS buffer (pH 7.4), then 5 mL of AuNCs (5 mg/mL) was added and the mixed solution was stirred at room temperature for 24 h. After that, the mixture was centrifuged and washed to remove the unbound AuNCs. In the next step, anti-HER2 was chemically modified on the surface of MSN-AuNCs. Briefly, EDC (4 mg) and Sulfo-NHS (6 mg) were added into above MSN-AuNCs solution and stirred for 6 h at room temperature. After removing the unreacted reactants by centrifugation and wash, 5 µmol anti-HER2 was added to the carboxyl-activated MSN-AuNCs. The resulting solution was stirred for 24 h. The free antibodies were removed by centrifugation and repeated washing. To detect the density of HER2 antibody on MSN-AuNCs, phycoerythrin-conjugated HER2 antibody (PE-anti-HER2) was linked to MSN-AuNCs instead of anti-HER2. The excess PE-anti-HER2 was monitored by the fluorescence test.

**Cell culture**
All the breast cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA). MCF10A cells were cultured in DMEM/F12 medium supplemented with 5% horse serum, 20 ng/mL of epidermal growth factor, 0.5 mg/mL of hydrocortisone, 100 ng/mL cholera toxin, 10 mg/mL of insulin and 1% penicillin/streptomycin. Hs578T cells were incubated in DMEM containing 10% FBS and 10 µg/mL of insulin. SKBR3 cells were cultured in McCoy5a medium containing 10% FBS and 1% penicillin/streptomycin. MDA-MB-436 and MDA-MB-231 cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. HCC1806 and MDA-MB-468 cells were cultured in RPMI-1640 supplemented with 10% FBS and 1% penicillin/streptomycin. MDA-MB-157 cells were incubated in L-15 containing 10% FBS and 1% penicillin/streptomycin. All cell lines were incubated at 37 °C in a 5% CO₂ humidified incubator.

**Cytotoxicity assay**
The cytotoxicity of MSN-AuNCs-anti-HER2 was evaluated by MTT assay. The SKBR3 cells were seeded in 96-well plates at a density of 5000 cells per well (100 µL) for 24 h. Then, the culture medium was replaced with fresh medium containing MSN-AuNCs-anti-HER2, followed by changing the medium back to complete medium after 2 h. The cells were subjected to MTT assay after another 24 h incubation. The absorbance values of the solution at 490 nm were measured with the microplate reader.
**Bioassay**
The enzymatic activity of MSN-AuNCs-anti-HER2 for oxidation of TMB was performed as follows: \( \text{H}_2\text{O}_2 \) (final concentration of 50 mM), TMB (final concentration of 800 μM) and different amounts of MSN-AuNCs-anti-HER2 were mixed in the phosphate buffer (25 mM, pH 4.0). Then the catalytic activities of mixtures were detected 30 min after the MSN-AuNCs-anti-HER2 was added.

**Detection of breast cancer cells**
After cells were centrifuged and rinsed with PBS, MSN-AuNCs-anti-HER2 was added to the cell suspensions and incubated for 90 min. Afterward, the above mixture was centrifuged and rinsed 3 times with PBS (pH 7.4), and then dispersed in detection buffer solution. Afterwards, \( \text{H}_2\text{O}_2 \) (final concentration 50 mM) and TMB (final concentration 800 μM) were introduced into the above mixed solution. The catalytic activities of the mixtures were detected after 30 min reaction.

**HER2-positive breast cancer tissue detection**
For efficient disaggregation of the tissues, the breast tumor samples (HER2 expression level: 3+/2+/1+/0) were firstly digested in a solution of collagenase, hyaluronidase and DNAse at 37 °C for 30 min with intermittent shaking.\(^5\) The obtained cell suspensions after filtering were rinsed 3 times with PBS. Then different cell samples from tissues (20000 cells) were incubated with MSN-AuNCs-anti-HER2 for 90 min. The cell samples were washed thrice with PBS and dispersed in the detection buffer solution. Then \( \text{H}_2\text{O}_2 \) (final concentration of 50 mM) and TMB (final concentration of 800 μM) were introduced into the above mixed solution. The catalytic activities of the mixtures were detected after 30 min reaction.
**Fig. S1** The zeta potentials of MSNs, amino-functionalized MSNs, carboxyl-functionalized MSNs.

**Fig. S2** The size distribution histogram of AuNCs. 100 particles were randomly selected from the TEM image from Fig. 1a for measuring the size distribution.
Fig. S3 TEM image and size distribution histogram of MSN. The total number of particles counted for the histogram was 90.

Fig. S4 N$_2$ adsorption-desorption isotherms and corresponding pore-size distribution curve (inset) of MSN.
Fig. S5 The EDS spectra of (a) MSNs, (b) amino-functionalized MSNs, (c) carboxyl-functionalized MSNs and (d) MSN-AuNCs-anti-HER2.

Fig. S6 Testing the ratio of AuNCs and MSN in MSN-AuNCs-anti-HER2. (a) The fluorescence spectra of AuNCs with known concentrations. The concentration of lysozyme was used to represent the concentration of AuNCs. (b) The linear relationship between fluorescence intensity at 660 nm and the concentration of AuNCs. (c) Fluorescence spectra corresponding to the AuNCs taken from the original solution (red line) and the supernatant after incubation with the MSN (green line). The ratio of AuNCs : MSN is calculated to be ~202 : 1. Inset: The fluorescence image of the MSN-AuNCs after high-speed centrifugation. The red fluorescence indicates the successful loading of AuNCs to MSN.
Fig. S7 (a, b) Calibration curves for the fluorescence intensity versus corresponding concentrations of PE-anti-HER2, showing a linear relationship between the fluorescence intensity at 577 nm and the concentration of PE-anti-HER2. (c, d) The conjugation of MSN with PE-anti-HER2. (c) The red line represents the original fluorescence of PE-anti-HER2, and the green line represents the remaining fluorescence with the corresponding centrifugal supernatant solution of PE-anti-HER2 after being conjugated with MSN. (d) The red histogram represents the fluorescence intensity of original PE-anti-HER2, and the green histogram represents the remaining fluorescence intensity with the corresponding centrifugal supernatant solution of PE-anti-HER2 after being conjugated with MSN. Data are presented as means ± SD (n = 3). First, the equation as follows is used to calculate the number of nanoparticles per gram of MSNs: 

\[ N = \frac{1}{m_{\text{SiO}_2}} = \frac{1}{(\rho_{\text{SiO}_2} V_s)} = \frac{1}{(\rho_{\text{SiO}_2} \times 4\pi r^3(1-V_p/(V_p+V_s)/3),} \]

where \( N \) is the number of nanoparticles per gram of MSNs, \( \rho_{\text{SiO}_2} \) is the density of MSN nanoparticles (\( \rho_{\text{SiO}_2} = 2.2 \text{ g/cm}^3 \)), \( r \) is MSN nanoparticle radius, \( V_p \) is pore volume, \( V_s \) is the solid silica volume (\( V_s = m/\rho_{\text{SiO}_2} = 1/2.2 = 0.455 \text{ cm}^3 \)). The number of MSN nanoparticles per gram is \( 2.39 \times 10^{17} \) in average. Then the ratio of antibody : MSN (\( N_{\text{antibody}}/N_{\text{MSN}} \)) can easily be calculated following the formulation: 

\[ N_{\text{antibody}}/N_{\text{MSN}} = \frac{(C_{\text{antibody}} \times V_{\text{antibody}} \times N_A)/(2.39 \times 10^{15})}{}, \]

where \( C_{\text{antibody}} \) is the molar concentration of PE-anti-HER2 linked to MSN, \( V_{\text{antibody}} \) is the solution volume of PE-anti-HER2, \( N_A \) is the Avogadro number (it is equal to \( 6.02 \times 10^{23} \)). Therefore, the average antibody density was about \( 232 \pm 12 \text{ molecules/MSN} \).
**Fig. S8** The peroxidase-mimicking activity of MSN-AuNCs-anti-HER2 as a function of incubation time. Data are presented as means ± SD (n = 3).

**Fig. S9** The H$_2$O$_2$ concentration dependent catalytic activity of horseradish peroxidase (HRP) and MSN-AuNCs-anti-HER2. Data are presented as means ± SD (n = 3).
**Fig. S10** *In vitro* cytotoxicity of MSN-AuNCs-anti-HER2 against SKBR3 cells. Data are presented as means ± SD (n = 3).

**Fig. S11** (a) Comparison of the catalytic activity of MSN-AuNCs-anti-HER2 incubated with the HER2\(^+\) breast cancer cells in supernatant from the third wash and the blank sample. (b) Comparison of the enzyme activity of MSN-AuNCs-anti-HER2 with or without centrifugation (1000 rpm, 5 min) in the absence of cells. Data are presented as means ± SD (n = 3). ns: no significant difference (P > 0.05). Statistical analysis was performed using Student's t-test.
Fig. S12 AuNCs-anti-HER2 for HER2+ breast cancer cell detection. (a) The absorbance spectra upon sensing of SKBR3 cells. (b) Plot of the absorption values at 652 nm versus different numbers of SKBR3 cells; the inset shows a linear relationship in the range from 200 to 5000 cells. Data are presented as means ± SD (n = 3). The detection limit is 200 cells.
References