A sensitive lateral flow test strip based on silica nanoparticle/CdTe quantum dot composite reporter probes

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Electronic Supplementary Information

(Including Experimental details, Optimization of sensing conditions, Supplementary figures and scheme)

Experimental details:

1 Reagents and material

Mouse anti-AFP monoclonal antibody$_1$ (anti-AFP mAb$_1$), mouse anti-AFP mAb$_2$, and AFP were obtained from Jingtian Biotechnology Limited (Shanghai, China). Bovin serum albumin (BSA), goat anti-mouse IgG, N-hydroxy-succinimide (NHS), and 3-aminopropyltriethoxysilane (APTES) were purchased from Sigma-Aldrich (St. Louis, USA). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was purchased from Fluka (Buchs, Switzerland). Glass fiber 8964 and spunbonded polyester 6613 were purchased from Ahlstrom (Helsinki, Finland). Nitrocellulose membrane M135 was purchased from Millipore (Temecula, USA). Other reagents were of analytical purity, and doubly distilled water was used for all experiments.

2 Preparation of colloidal gold-mAb$_1$ probes

The gold nanoparticles were synthesized using the method of Frens [1, 2] and were used as reporters in gold-based test strips. Before conjugating colloidal gold with mouse anti-AFP-mAb$_1$, the optimum pH and the minimum amount of mAb$_1$ for stabilizing the colloidal gold solution were determined as described previously [3]. For conjugation, 2.3 ml of mouse anti-AFP-mAb$_1$ solution (0.1 mg/ml) was added drop by drop into 10 ml of colloidal gold solution under magnetic stirring. After purification, the resulting pellets were resuspended in 1 ml of the phosphate buffer solution (PBS, 1 mM, pH7.4).

3 Preparation of silica/CdTe composite probes

The water-soluble CdTe QDs were prepared based on the reaction between $CdCl_2$•$2.5H_2O$ and NaHTe solution in the presence of mercaptopropinic acid (MPA) as a stabilizer [4, 5].

The silica nanoparticles were prepared using the inverse microemulsion method as previously described (Wang, 2009) with some modifications. A water-in-oil reverse microemulsion was formed by mixing 37.5 ml of cyclohexane, 9 ml of n-hexanol, 8.85 ml of Triton X-100, and 2.5 ml of water. Then, 0.5 ml of tetraethoxysilane (TEOS) and 0.3 ml of ammonium hydroxide (25%) were added into the microemulsion system sequentially to initiate the hydrolysis. The resulting product was vacuum-dried. APTES was utilized to amino-modify silica nanoparticles [6] for their further modifications.

The EDC and NHS were used as crosslinkers to conjugate the amino-modified

silica nanoparticles with mouse anti-AFP mAb_1 and MPA-capped CdTe QDs. In brief, 20 mg of the amino-modified silica nanoparticles were suspended in 10 ml of borate buffer solution (BBS, 1 mM, pH=9.0), then, 0.225 ml of EDC (1 mg/ml) and 0.3 ml of NHS (1 mg/ml) were added, followed by addition of 0.3 ml of mouse anti-AFP mAb_1. After blending by vortex for 1 min, the mixture was incubated at 37 ºC under gentle shaking for 2 h. Then the conjugates were collected by high-speed centrifugation (two sequential spins at 14,000g, 20 min). Next, the pellets containing antibody conjugated silica nanoparticles were resuspended in 10 ml of BBS, followed by the sequential addition of 1 ml of EDC (1mg/ml), 1.3 ml of NHS (1mg/ml) and 3 ml of CdTe QDs. The mixture was incubated at 37 ºC under shaking for 2 h, followed by centrifugation at 14 000g for 20 min to remove the unbound CdTe QDs. Finally the silica/CdTe-mouse anti-AFP mAb_1 conjugates (pellet) were resuspended in the BBS containing 1% BSA, 4% sucrose, 0.05% Tween-20, and 0.05% sodium azide. The composite reporter probes were stored at 4 ºC for the further experiments. The ratios of EDC\NHS and antibodies were according to our previous researches[7]. A schematic diagram of the complete procedure for synthesis of the composite probes is given in Scheme 1.

4 Fabrication of lateral flow test Strips

As shown in Fig.S4, the test strip consisted of a nitrocellulose membrane containing an anti-AFP mAb_2 test line and an anti-mouse IgG control line, a conjugate pad containing the anti-AFP mAb_1: silica nanoparticle/CdTe composite QD reporter probe, a sample pad, and an absorbent pad. All of these parts were pasted on a black

backing plate. Then the whole assembled test strip was cut into 4 mm strips and stored under dry conditions at room temperature.

Supplementary figures and scheme:

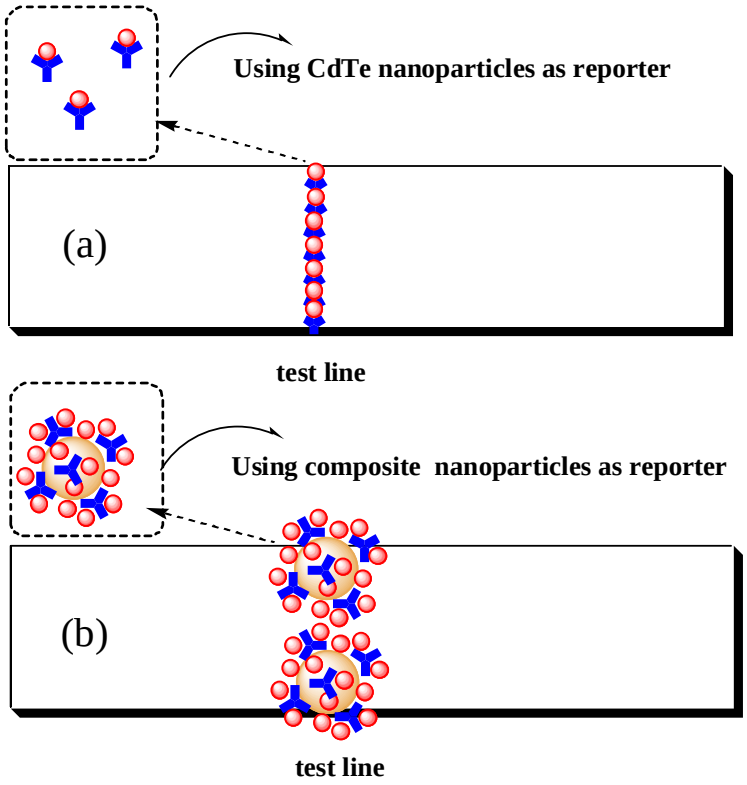

Fig. S1 Schematic diagram of a lateral flow test strip using (a) CdTe nanoparticles and (b) composite nanoparticles containing a large number of CdTe QDs as reporter

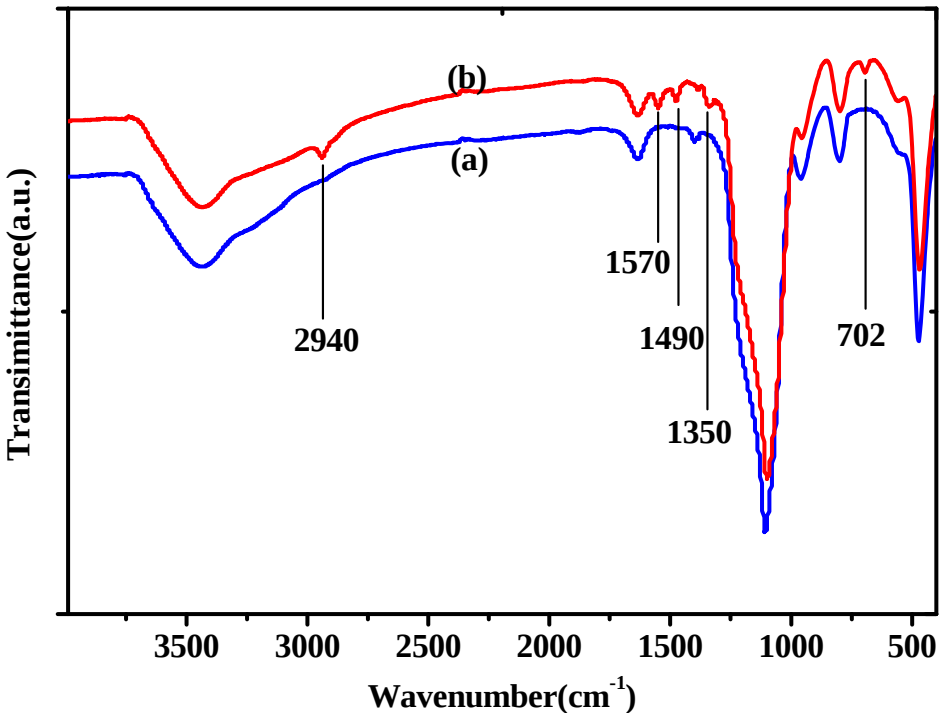

Fig. S2 FT-IR spectra of (a) the silica particles and (b) the amino-modified silica particles.

Amino-modified silica nanoparticles present characteristic absorption bands at 2940 cm^{-1}, 1570 cm^{-1}, 1490 cm^{-1}, 1350 cm^{-1}, and 702 cm^{-1} compared with silica nanoparticles. The bands around 2940 cm^{-1} and 1350 cm^{-1} are attributed to C-H and C–N stretching vibration, respectively. The bending vibration of C-H appears at 1490 cm^{-1}. The deformed and bending vibration of N-H are observed at 1570 cm^{-1} and 702 cm^{-1}. These results indicated that APTES has been bonded onto the surface of the silica nanoparticles through a silanization reaction.

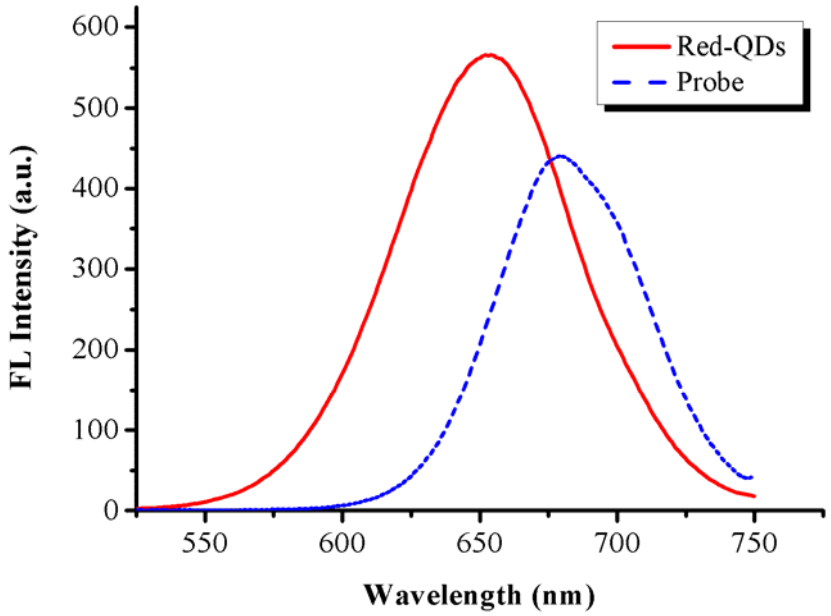

Fig. S3 The fluorescent emission spectrum of Red-CdTe QDs and composite reporter probes (excited at 375 nm)

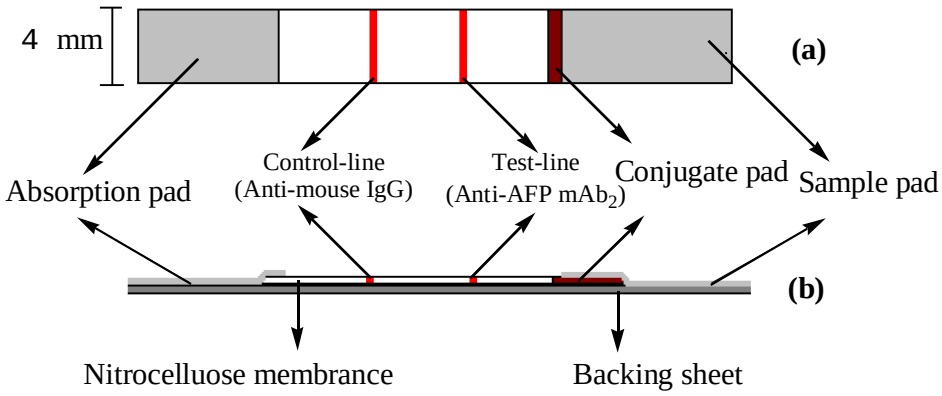

Fig. S4 Schematic diagram of the immunochromatographic lateral flow test strip (a): top view. (b): cross-section (Conjugate pad contains the anti-AFP mAb_1:silica nanoparticle/CdTe composite QD reporter probe)

References

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