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Quantum-dot-tagged photonic crystals beads for multiplex detection of tumor markers

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Experimental

Materials and reagents: Human AFP and CEA, mouse monoclonal anti-human AFP and anti-human CEA antibodies, Alexa Fluor 350 (AF350) labeled goat anti-human AFP and anti-human CEA antibodies were obtained from Beijing Biosynthesis Biotechnology Co., LTD. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-Hydroxysulfosuccinimide sodium salt (Sulfo-NHS), bovine serum albumin (BSA) and 2-(N-morpholino)-ethanesulfonic acid (MES) were purchased from Sigma Chemicals. Oil-soluble CdSe/ZnS QDs (central photoluminescence peaks at 545 nm and 615 nm) were purchased from Wuhan Jiayuan Quantum Dots Co., Ltd. Clinical serum samples were from Zhongda Hospital Southeast University, China. Phosphate buffer saline (PBS, 0.05 M, pH 7.0), phosphate buffer saline tween-20 (PBST, 0.05% tween-20 in PBS) and MES buffer (0.1M, pH 5.5) were self-prepared. All buffers were prepared with water purified in a Milli-Q system (Millipore, Bedford, MA).

Instruments: Bright-field and fluorescence images were obtained with an inverted fluorescence microscope (Olympus IX73) and a CCD camera (Media Cybernetics Evolution MP 5.0). Reflection and fluorescence spectra of QD-tagged PCBs were recorded by an optical microscope equipped with a fiber-optic spectrometer (Ocean Optics, USB2000). A longpass dichroic filter (DM 400, Chroma Technologies,

Brattleboro, VT) was used to reject the scattered light and to pass the Stokes-shifted fluorescence signals. The microstructures of the QD-tagged PCBs were characterized by scanning electron microscopy (SEM; Hitachi, S-4800)

Synthesis of polystyrene nanospheres: Monodisperse polystyrene (PS) nanospheres with diameters ranging from 180 to 320 nm were synthesized by the soap-free emulsion method.[S1] Typically, 20 mL styrene monomer and 200 mL water were added to the three-neck flask with a stirring speed of 300 rpm. The mixture was raised to reflux for 3 min, and 0.2 g potassium persulfate powder was then added to the solution. After reaction for 2 h, the monodisperse PS spheres could be obtained. Their diameter could be tuned by changing the amount of styrene monomer.

Synthesis of QD-tagged PCBs: Fluorescent PS nanospheres were firstly prepared by swelling the PS nanospheres in a solvent mixture containing 5% (vol/vol) chloroform and 95% (vol/vol) hexane, and then adding a controlled amount of CdSe/ZnS QDs to the mixture. And then resultant fluorescent nanospheres self-assembled into QD-tagged PCBs using a microfluidic device[S2]. Briefly, an aqueous suspension containing monodisperse fluorescent PS nanospheres was used as the aqueous phase. The oil phase consisted of poly(dimethylsiloxane) and poly-(1,1,1-trifluoropropylmethylsiloxane) (7:3 in volume). The oil phase and aqueous suspension were simultaneously injected into the PTFE tube at the speed of 30 and 1 mL/h, respectively. At this time, the aqueous suspension was broken into droplets by the oil flows at the needle tip. The suspension droplets were delivered into the collection container filled with the silicon oil by the oil flow. Next, the collection container was placed in an electrothermal oven and maintained at 60 °C for12 h. The nanoparticles could self-assemble into ordered lattices during the evaporation of water in the droplets. After the solidification, the obtained QD-tagged PCBs were thoroughly washed with hexane to remove the silicon oil.

Immobilization of antibodies onto QD-tagged PCBs: Antibody probes were covalently attached to the surface of carboxyl groups activated QD-tagged PCBs by two-step carbodiimide method.[S3,S4] The prepared QD-tagged PCBs (~100 spheres) were resuspended in 100 μL MES buffer, and 5 mg EDC and 5 mg NHS were then added into the buffer to activate the carboxyl groups for 2 h. After the activated beads were washed with PBS buffer for three times, 100 μL of antibody probes (100 μM in

PBS buffer) was added to the suspension, and reacted for 2 h at room temperature under shaking and finally kept overnight at 4°C. After washed with PBS buffer for three times, the nonspecific sites on the QD-tagged PCBs surface were blocked using 1% BSA in PBS buffer.

Multiplex Detection of tumor markers: For multiplexed detection of tumor markers, two kinds of QD-tagged PCBs were immobilized with anti-human AFP antibody and anti-human CEA antibody, respectively. And then these two kinds of carrier-antibody conjugates were mixed in one test tube, and a mixture of AFP and CEA tumor markers and AF350-labeled anti-human AFP and anti-human CEA antibodies were added. The mixture was incubated for 30 min under shaking. After the QD-tagged PCBs were washed with 1% BSA PBST buffer for three times, the fluorescence spectra and reflection spectra of beads were measured using a inverted fluorescence microscope equipped with a fiber-optic spectrometer. The fluorescence signals for AFP and CEA could be distinguished and detected by use of their code signals for AFP and CEA, respectively. The number of replicates at the any concentration was 5.

The optical microscopy image of QD-tagged PCBs

Fig. S1 The optical microscopy image of QD-tagged PCBs.

The cross-reactivity for the multiplex immunoassay of two tumor markers

Fig. S2 The cross-reactivity for the multiplex detection of AFP and CEA, which was performed by comparing the fluorescence signals at 10 ng/mL concentration of specific analyte with increasing levels of the other coexistent analytes, respectively (n=5 for each point).

Table S1 Assay results of clinical serum samples using the proposed and reference methods (ng/mL)

	AFP			CEA		
sample	proposed method	reference method	relative error (%)	proposed method	reference method	relative error (%)
1	2.2	2.3	-4.3	1.2	1.3	-7.7
2	4.8	4.5	6.7	1.7	1.6	6.3
3	5.5	5.2	5.8	4.2	3.9	7.7
4	6.8	6.2	6.5	6.7	6.2	8.1
5	6.9	6.4	7.8	6.9	6.4	7.8
6	6.9	7.0	-1.4	9.1	8.4	8.3
7	11.1	10.2	8.8	23.5	25.3	-7.1
8	62.3	57.3	8.7	48.8	50.6	-3.6
9	84.3	84.6	-0.4	79.0	73.7	7.2
10	229	212	8.0	640	590	8.5

References

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