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Biofunctionalized mesoporous silica nanospheres for ultrasensitive chemiluminescent immunoassay of tumor marker

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Experimental

Materials and Reagents: AFP ELISA reagent kit was bought from CanAg diagnosics, and includes *a s*eries of AFP standard solutions from 0 to 500 ng/mL, the stock solution of horseradish peroxidase (HRP)-labeled mouse monoclonal anti-AFP (20 μg/mL) and biotinylated mouse monoclonal anti-AFP (1.0 μg/mL) .Streptavidin was bought from Promega Corporation. Sixteen alkyl three methyl bromide (CTAB), triethoxysilane (TEOS), and hydrogen peroxide (30%, H_2O_2) were obtained from Sinopharm Chemical Reagent Co (China). *γ*- Glycidoxypropyltrimethoxysilane (GPTMS, 98%), chitosan and bovine serum albumin (BSA) were obtained from Sigma (Louis St., MO). Luminol (Acros, Belgium) stock solution (0.01 M) was prepared in 100 mL of 0.1 M NaOH. P-iodophenol (PIP, Alfa Aesa China Ltd.) stock solution (0.01 M) was prepared by dissolving 110 mg of PIP in 5 mL dimethylsulfoxide and diluting to 50 mL with distilled water. Prior to use, luminol and PIP stock solutions were mixed and diluted with 0.1 M pH 8.5 Tris-HCl buffer. CL substrate for enzymatic CL reaction is comprised of 5.0 mM luminol, 0.6 mM PIP and 4.0 mM H_2O_2. The coupling buffer used for antibody immobilization is 0.01 M pH 7.4 phosphate buffer, blocking buffer is phosphate buffer containing 1% BSA, and wash

buffer used to minimize unspecific adsorption is prepared by spiking 0.05% Tween-20 into 0.01 M pH 7.4 phosphate buffer.

Apparatus: Flow-through CL immunoassay device is shown in Figure S1 (Supporting Information). A thin-layer flow cell as an immunoreactor (Figure S1a) is composed of a Teflon cover (4.0 cm × 2.5 cm × 0.8 cm) with inlet and outlet, a silicon slice rubber spacer (2.0-mm thickness), and a transparent plexiglass slice (4.0-cm thickness). The antibody-immobilized glass slide (2.5 cm × 0.3 cm × 0.1 cm) is fixed on the center area of inner side of Teflon cover. The total thickness of the antibody-immobilized slide and film is about 1.1 mm. The volume of the constructed cell is about 60 μL (2.2 cm × 0.3 cm × 0.09 cm). Teflon tubes (0.8 mm i.d.) and silicon rubber tubes (1.0 mm i.d.) are used to connect all the components of the flow-through assay system (Figure S1b). All solutions are carried with a multichannel peristaltic pump. A multiposition valve with five inlets and one outlet is used to sequentially switch different solutions into the flow-through assay system. The flow cell is positioned in front of photomultiplier (PMT) of an IFFM-E luminescence analyzer (Remex Analytical Instrument Co. Ltd., Xi'an, China). The CL emission is measured with the PTM operated at –800 V. Instrument control and data recording were performed using IFFM software.

The reference electrochemiluminescence immunoassay was obtained with a Roche Elecsys 2010 immunoassay analyzer (Roche Diagnostics GmbH). Scanning electron microscopy (SEM) images were obtained with a Hitachi S-4800 scanning electron microscope (Japan) at an acceleration voltage of 15 kV. Transmission electron microscopy (TEM) image was obtained with a Philips Tecnai-12 transmission electron microscope (Holland) at an acceleration voltage of 100 kV. The static water contact angles were measured with a contact angle meter (Rame-Hart-100) using droplets of distilled water at room temperature (RT, 25 °C).

Preparation of MCM-41-type MSNs and AFP immunosensor: MCM-41-Type MSNs were synthesized according to previous literature with some modifications.[27] 1.0 g of CTAB was dissolved in 480 mL of distilled water, and 3.50 mL of sodium hydroxide solution (2.00 M) was added to above-

mentioned solution. The temperature of the resultant solution was controlled at 80 °C. 5 mL of TEOS was added dropwise to the solution, and the mixture was stirred at 80 °C for 2 h. The resulting white precipitate was separated by filtration, and then calcined in a muffle furnace at 550 °C for 5 h. Finally, the silica particles were remove the template to form mesoporous silica nanospheres.

Next, glass slides were firstly immersed in piranha solution (H_2SO_4/30% H_2O_2, 7:3 in volume) for 12 h. After washing thoroughly with distilled water, they were dried under a stream of nitrogen, and then silylanized by immersing them in 1% GPTMS toluene solution overnight at RT. Afterwards, the epoxy-activated glass substrates were rinsed three times with pure toluene and ethanol to remove the physically absorbed GPTMS and dried under a stream of nitrogen. 2.0 mg of MSNs were dispersed in 1.0 wt% chitosan solution with ultrasonication. The resultant suspension was mixed with 100 μg/mL of streptavidin at 1: 1 volume ratio. After stirring for 10 min, 20 μL resulting mixed solution was dropped on the epoxy-activated glass slide for the reaction for 1 h at RT, and followed at 4 °C overnight. After washing several times with wash buffer, and blocked with blocking buffer for 12 h at 4 °C, 20 μL of biotinylated monoclonal anti-AFP (1.0 μg/mL) was dropped on the surface of streptavidin-functionalized glass substrate and reacted at RT for 3 h. The immunosensors were kept in refrigerator at 4 °C prior to use.

CL immunoassay procedure: The flow-through CL immunoassay process for AFP was shown in Table S1. The mixture of 30 μL sample and 30 μL of 1.0 μg/mL HRP-labeled anti-AFP were firstly delivered into the flow cell and incubated under stop flow at RT for 20 min. Wash buffer was then introduced into the flow-through assay system at an optimal flow rate of 1.0 mL/min to wash the immunoreactor. CL substrate was carried to the flow cell to trigger the enzyme-catalyzed CL reaction for 30 s, and CL signals were measured by PMT. The whole procedure from sample injection to signal collection can be completed within 24 min.

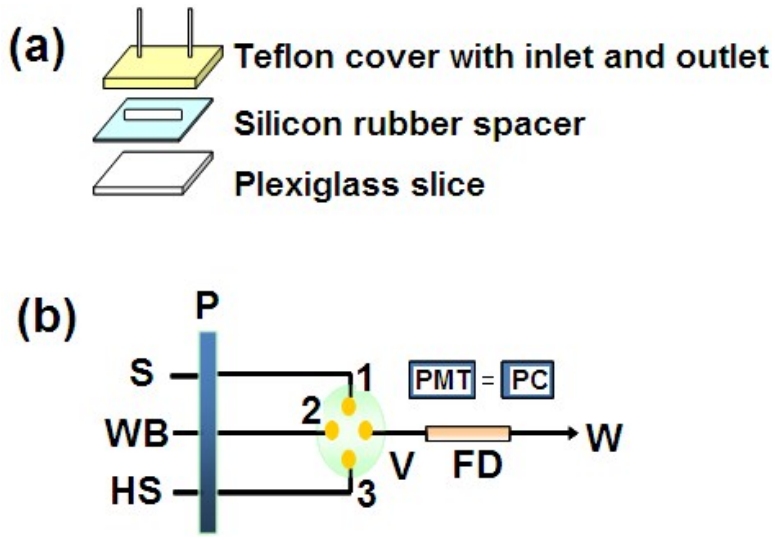

Figure S1. Scheme of flow-through CL immunoassay system including (a) thin-layer flow device, and (b) flow-through assay system: (S) sample, (WB) wash buffer, (HS) HRP substrate, (V) multiposition valve, (P) peristaltic pump, (FD) flow device, and (W) waste solution.

Table S1 Detailed process for the flow-though CL immunoassay of AFP

Step no.	Valve position	step	Starting time (min:s)
1	1	introduce the mixture of AFP sample and AFP tracer into the flow device	00:00
2	1	stop flow and incubation at room temperature	00:30
3	2	wash the flow device with wash buffer at a flow rate of 1.0 mL/min	20:30
4	3	introduce 60 μL CL substrate into the flow device and stop flow to collect data	22:30
5	1	introduce wash buffer to wash flow device at 1.0 mL/min and renew the immunosensor	23:00
6	1	ready for the next assay cycle	24:00

References

S1 Z.X. Li, J.C. Barnes, A. Bosoy, J.F. Stoddart, J.I. Zink, *Chem. Soc. Rev.*, 2012, **41**, 2590–2605.