Fabrication of Multifunctional Magnetic Nanogold Microspheres as Immunosensing Probe for the Detection of Staphylococcal Enterotoxin B in Food

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This research describes the fabrication process of multifunctional magnetic nanogold microspheres as immunosensing probe for the detection of staphylococcal enterotoxin B, as a model, in food.

The emerging research field of nanotechnology, the process to generate, manipulate, and deploy nanomaterials, provides exciting possibilities for advanced development of new analytical tools.[1] One major merit of using nanomaterials is that one can control and tailor their properties in a very predictable manner to meet the needs of specific applications. Magnetic beads have been highly attractive as an ideal host owing to high chemical and thermal stability, fine suspendability in aqueous solution, and good compatibility with the environment.[2,3] Protein-mediated assembly of magnetic beads is a potent tool for fabrication of new materials, which combine tunable nanoparticle features (size, surface functionality, and core properties) with the unique physical and chemical properties of protein and peptides.[4,5] The approach, however, has been limited to the modification of the nanoparticles to directly react with protein-recognition sites. An alternative strategy would be to target the surface of the protein through complementary interactions, using the shape and physical characteristics of the protein to dictate the structural feature in the resulting nanoparticle and protein composites.

Herein we initially prepared multifunctional magnetic nanogold microspheres (MMSs) by using magnetic Fe_3O_4 nanoparticle as core and thionine-bound gold nanoparticles as shell, and then the prepared MMS was used as an affinity support for the conjugation of staphylococcal enterotoxin B-bound bovine serum albumin (SEB-BSA), as a model (Figure 1a). With an external magnet, the SEB-BSA-labeled MMSs were attached on the surface of a gold electrode. A competitive immunoassay format was employed to detect SEB with horseradish peroxidase (HRP)-labeled anti-SEB as tracer and hydrogen peroxide as enzyme substrate. When the prepared MMSs were incubated in a mixed solution containing HRP-anti-SEB and SEB sample for 1 h at 37 °C, the amperometric response decreased with the increment of SEB sample concentration. The detection process is illustrated in Figure 1b.

Prior to experiments, we initially prepared 20-nm Fe_3O_4 nanoparticles and 8-nm gold colloids according to our previous reports, respectively.[6,7] The fabrication of the MMSs was as follows (Figure 1a): 0.5 g of Fe_3O_4 nanoparticles was initially added into 20 mL of 0.5 M NaCl solution containing 0.025 M sodium dodecyl sulfate (SDS) and stirred for 4 h. After washing with water, the SDS–Fe_3O_4 composites were redispersed in 20 mL of 0.2 M acetate buffer (pH 3.5). 2 mL of chitosan (0.5%, w/w) in 1% acetatic acid was added and stirred for 8 h. (*Notes*: The excess SDS and chitosan were removed in the supernatant fraction after centrifugation). After washing three times with 0.002 M acetate buffer (pH 3.5) and ultrapure water alternately, the chitosan–Fe_3O_4 composites were cross-linked with 2.5% glutaraldehyde (pH 4.0) at room temperature for 4 h. The products were enriched with the aid of an external magnet. Afterward, the chitosan–Fe_3O_4 nanocomposites obtained were added to 8-nm gold colloids and slightly shaken for 4 h at room temperature to make gold nanoparticles assemble on the surface of the formed nanocomposites. Finally, the formed MMSs were obtained by magnetic separation and stored at 4 °C when not in use. Figure 1c shows the TEM image of the as-prepared MMSs.

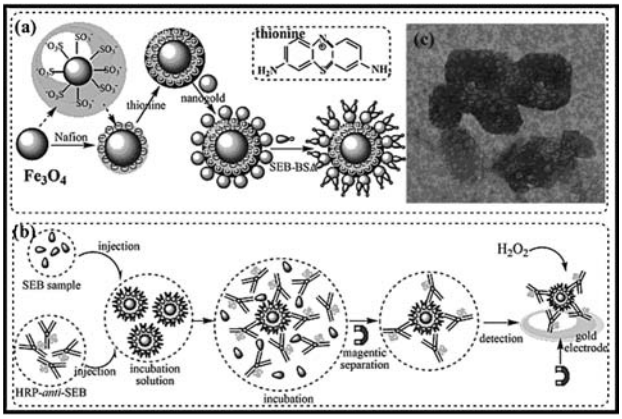

Figure 1. (a) Schematic representation of the multifunctional magnetic nanogold microspheres, (b) schematic view of measurement method, and (c) TEM image of the multifunctional magnetic nanogold microspheres.

To investigate the performance of the MMSs, the prepared MMSs were employed for the conjugation of SEB-BSA, as a model protein. Prior to experiments, we used a quartz crystal microbalance (QCM) to evaluate the immobilized amount of SEB-BSA on the MMS surface according to the Sauerbrey equation ($\Delta f_x = -2.3 \times 10^{-6} f_0^2 \Delta m/A$).[8] The immobilized amount of SEB-BSA was calculated according to the mass change before and after the SEB-BSA immobilization on the MMS surface. For comparison, we also prepared core–shell gold–Fe_3O_4 nanoparticles for the immobilization of SEB-BSA. Experimental results are shown in Figure 2. Seen from Figure 2, the probe with the MMS particles exhibits more frequency shift than that of core–shell gold–Fe_3O_4 nanoparticles toward the same concentration of SEB-BSA; that is, the surface coverage of biomolecules on the MMSs could be improved.

Following that, a cyclic voltammetric measurement with a competitive immunoassay format was employed to detect SEB with HRP-labeled anti-SEB as tracer and H_2O_2 as substrate. The current shift increased with the increment of SEB concen-

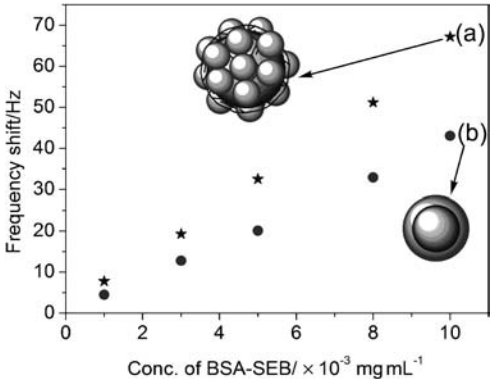

Figure 2. Frequency responses of various probes toward different concentrations of SEB-BSA: (a) multifunctional magnetic nanogold microspheres and (b) core–shell gold–Fe_3O_4 nanoparticles (*Note*: Adsorption time: 60 min at room temperature).

tration in the sample solution before and after the antigen–antibody interaction in the H_2O_2–PBS system (pH 6.5). The current shift was proportional to SEB concentration in the range of 0.5 to 12 ng/mL and the linear regression equation is i_{pc} (μA) $= 0.799 + 1.306 \times C_{[SEB]}$ (ng/mL) with a detection of 0.05 ng/mL (Figure 3a) at a signal-to-noise ratio of 3δ (where δ is the standard deviation of a blank solution, $R^2 = 0.998$). For comparison, we also used the core–shell gold–Fe_3O_4 nanoparticles as probe for the detection of SEB by using the same protocols, and the linear ranges and detection limits are 1.5–10.0 ng/mL with a detection of 0.5 ng/mL SEB (Figure 3b). It revealed that the prepared MMSs possessed the advantages of higher sensitivity and wider linear range compared with that of core–shell gold–Fe_3O_4 nanoparticles as probe.

The reproducibility of the immunoassay system was evaluated by intra- and interassay coefficients of variation (CVs). The intra-assay precision of the analytical method was evaluated by analyzing four concentration levels five times per run. The CVs of intraassay with this method were 4.5, 6.7, 5.2, and 6.9% at 1.0, 3.0, 5.0, and 10.0 ng/mL of SEB, respectively. Similarly, the interassay CVs on five immunosensors were 6.3, 7.8, 5.8, and 6.1% at 1.0, 3.0, 5.0, and 10.0 ng/mL of SEB, respectively. Thus, the precision and reproducibility of the proposed immunoassay was acceptable. The stability of the immunoassay system was examined. When the prepared MMSs were stored at 4 °C, it retained 90.2% of its initial response after a storage period of 21 days.

To test for robustness of the method and occurrence of SEB, naturally contaminated peanut samples were obtained from the local market. The concentrations of these samples were assayed by the developed immunoassay method and the commercially

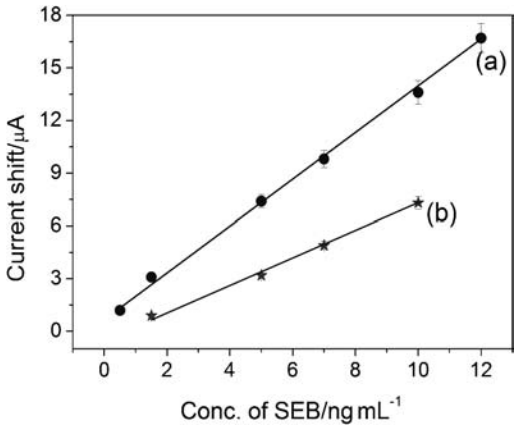

Figure 3. Calibration curves for the competitive immunoassay of SEB recorded by using various immunoprobes: (a) multifunctional magnetic nanogold microspheres and (b) core–shell gold–Fe_3O_4 nanoparticles.

available ELISA method, respectively. The regression equation (linear) for these data is as follows: $y = 1.5788 + 0.9434x$ ($R^2 = 0.9942$) (x axis, by the as-prepared immunoassay; y axis, by ELISA). These data show that there is no significant difference between the results given by the two methods, which are in accordance with the results obtained using the standard methods proposed by ELISA; that is, the developed immunoassay may provide a promising alternative tool for determining SEB in food.

In summary, the proposed immunoassay system with the multifunctional magnetic nanogold microspheres as immunosensing probe exhibited high sensitivity and good reproducibility. Although the present assay system is focused on the determination of the target toxin, it can be easily extend to the detection of other mycotoxins in food.

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