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Development a novel approach of chemiluminescent probe array

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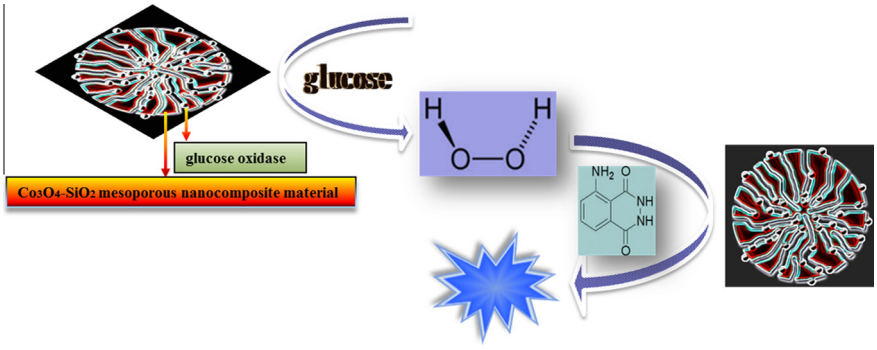
HIGHLIGHTS

- A novel CL probe array assay was first developed.
- It gives rapid and high throughput detection.
- It breaks traditional development view in solid phase supports.

GRAPHICAL ABSTRACT



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ABSTRACT

A new chemiluminescent (CL) probe array assay approach was first developed. The new CL probe array was based on Co_3O_4–SiO_2 mesoporous nanocomposite material, which not only has an excellent catalytic effect on the luminol–H_2O_2 CL reaction in alkaline medium but also can be used for the immobilization of enzymes. As a model, the novel bifunctional CL probe array has been applied to the high-throughput determination of glucose in human. The linear range of the glucose concentration was 3–90 μM and the detection limit was 0.36 μM. It breaks traditional development view in solid phase supports and provides new insights into the application of mesoporous material.

Introduction

Immobilized enzymes typically have greater thermal and operational stability than the soluble form of the enzyme [1–3]. Many different carriers have been studied for the immobilization of enzymes. They can be classified as inorganic and organic according to their chemical composition. Organic carriers include cellulose, dextran, agar, agarose, chitin, alginate, collagen, albumin, carbon, polystyrene, polyacrylatepolymethacrylates, polyamides, polyacrylamide, vinyl, and allyl-polymers [4,5]. Inorganic carriers include bentonite, glass, silica, metals, and metal oxides [6,7]. Inorganic carriers often display good mechanical properties, thermal stability, and resistance against microbial attack and organic solvents. However, non-porous materials like metal and metal oxides only have small binding surfaces. Mesoporous material is one of the most promising carriers for enzyme immobilization. The unique properties of mesoporous materials were huge surface area and restricted pore nanospaces. Furthermore, mesoporous materials offer nanostructure for enzymes that can be finely tuned through controlling their pore structure, transport and microenvironment [8–10]. Recently, some research groups have immobilized enzymes on mesoporous silica which showed improvement on enzyme stability, catalytic activity, products specificity, and

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resistance to extreme environmental conditions [11–13]. However, all of the present carriers only play one role of supporter.

Nanotechnology has provided new opportunities in many fields. One of the most important applications of nanoparticles is catalysis, where the large surface area per unit volume of nanosized catalysts enhances reactions. Recently, much attention has been extended to the CL of nanomaterial systems, to improve the sensitivity and the stability [14]. Our group has demonstrated that Co_3O_4 nanoparticles could enhance the luminol–H_2O_2 CL system [15–17]. Jia et al. reported a two-step method for the preparation of a Co_3O_4–SiO_2 mesoporous nanocomposite material that exhibits very high activity for CO oxidation even at a temperature as low as −76 °C [18,19].

Recently, bifunctionalized nanoparticles have gained great interest in the biomedical applications [20]. Bifunctional nanoparticles integrate two formerly distinct functionalities into a single entity with superior and sometimes unprecedented properties such as detection by multiple imaging modalities, or detection and therapy.

In this work, we report a bifunctional Co_3O_4–SiO_2 mesoporous nanomaterials entrapped function enzymes inside the nanochannel by a simple synthesis operation. The Co_3O_4–SiO_2 mesoporous nanomaterials are well dispersed with a diameter of 20–30 nm. Glucose oxidase (GOx) was chosen as a model enzyme to prepare the enzyme reactor. Meanwhile, the Co_3O_4–SiO_2 mesoporous nanomaterials exhibited higher specific catalytic effect on the luminol–H_2O_2 CL reaction in alkaline medium owing to its high specific surface area and mesoporous channels. The combination of excellent luminescent properties and enzyme reactor suggest a great promise in the application of these bifunctional nanoparticles in catalysis chemiluminescence and in biomedicine such as determination of glucose in serum (Scheme 1). To the best of our knowledge, this is the first example of the use of nanomaterial to plays two important roles of catalyzer of CL reaction and supporter. The new CL probe array has been successfully applied to high-throughput determination of glucose in human serum.

Experimental

Materials and methods

3-Aminophthalhydrazide (luminol) and glucose oxidase (GOx) were purchased from Sigma–aldrich (USA). Sodium hydroxide solution (NaOH), activated carbon, d-Glucose, Co $(NO_3)_2$, tetraethoxysilane (TEOS), and ethanol were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Millipore Milli-Q (18 MΩ cm) water was used in all experiments. All reagents were used without further purification. The 96-well plates were provided by JET BIOFIL products.

Instruments

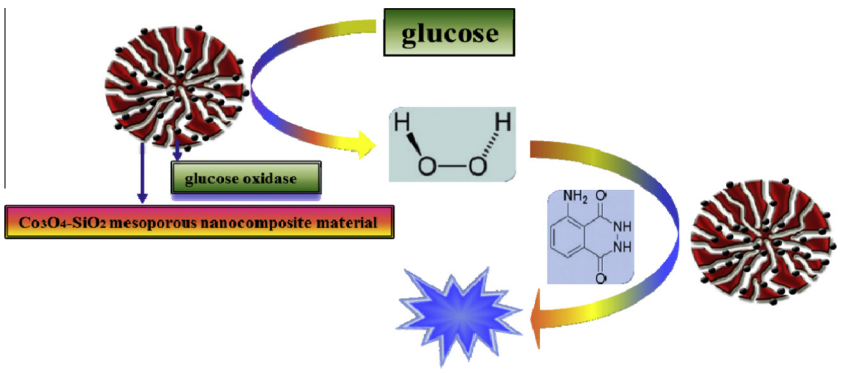
The Synergy™2 Multi-Mode Microplate Reader with the low-noise photomultiplier tubes (PMT) detector was used for the detection of luminescence. An external dispense module pumped fluid from the supply bottles to the two injectors located inside the instrument. Online monitoring of each probe can be computer-controlled via BioTek's Gen5 PC software. The transmission electron microscopy (TEM) images were obtained with JEM-2100 TEM (Hitachi, Japan). Scanning electron microscopy (SEM) images were obtained with Quanta 200 SEM (FEI, Japan). All of the nanoparticles were centrifuged on an HC-3018R centrifuge (Anhui, China). The CL spectrum was obtained using the modified Hitachi F-4600 spectrofluorimeter combined with a flow injection system. The surface area, pore size, and pore volume were determined by N_2 adsorption–desorption isotherms obtained at 77.156 K on a Micromeritics ASAP 2020M.

Preparation of Co_3O_4–SiO_2 mesoporous nanocomposite material entrapped function enzymes inside the nanochannel synthesised

The bifunctional Co_3O_4–SiO_2 mesoporous nanomaterials entrapped function enzymes inside the nanochannel synthesised referring to the procedure reported by Jia et al. with some change [19]. The 20 g of activated carbon was impregnated under magnetic stirring with 5.4 mL of TEOS diluted with 8 mL of ethanol. The solution was completely absorbed by the activated carbon in 5 min under continuous stirring. Then the composite material was transferred into a muffle oven and annealed at 350 °C for 30 min in air. The above C–SiO_2 composite material was added to 10.4 mL of 4.0 M Co $(NO_3)_2$ solution under magnetic stirring for 30 min. The resulting solid was transferred into a muffle oven and calcined at 550 °C for 90 min in air. Co_3O_4–SiO_2 mesoporous nanocomposite material was obtained. A 2-mL volume of 6 mg mL^{-1} GOx stock solution dissolved in 5 mM pH 6.5 tris (hydroxymethyl) aminomethane–HCl buffer was added to 15 mg Co_3O_4–SiO_2 mesoporous nanocomposite material. The mixture was stirred for 8 h at 4 °C. The Co_3O_4–SiO_2 mesoporous nanocomposite material-immobilized GOx was separated from solution by centrifugation and washed with distilled water.

Measurements of CL probe array

The Co_3O_4–SiO_2 mesoporous nanocomposite material-immobilized GOx was well dispersed in 10 mL distilled water, and then was added into each well (100 μL each probe). Glucose solution was added into the each probe (100 μL). The mixture was then incubated at room temperature for 20 min to yield the testing sample solution. A 50 μL volume of luminol solution were injected into



Scheme 1. The measurement principle of the glucose probe array.