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Fabrication of novel superoxide anion biosensor based on 3D interface of mussel-inspired Fe_3O_4-$Mn_3(PO_3)_2$@Ni foam

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ABSTRACT

Preparation of high performance electrochemical biosensing interface for the sensitive and rapid detection of human metabolites is of great interest for health care and biomedical science. In this paper, based on the adhesion technique of marine mussels, we designed and prepared a novel biosensor with a micro/nano-biointerface of Fe_3O_4-$Mn_3(PO_3)_2$@Ni foam, which offered a three dimensional (3D) living environment for real cell. The constructed biosensor with a 3D micro/nano-biointerface of Fe_3O_4-$Mn_3(PO_3)_2$@Ni foam was characterized by scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS) and elemental mapping. Furthermore, the electrochemical experiments by electrochemical method for detection of superoxide anion ($O_2^{\bullet -}$) in situ released by cells were carried out by this biosensor we proposed. Results indicated that the 3D interface of mussel-inspired Fe_3O_4-$Mn_3(PO_3)_2$@Ni foam offered an amicable platform for promoting cell adhesion, which was beneficial for enhancing biosensing activity. This proposed sensing platform provided high electroactivity and excellent electron transport with a lower detection limit (0.0170 μM), wider linear range 0.04–2.44 μM) and short diffusion distance to reaction sites. The case achieved the accurate detection of $O_2^{\bullet -}$ (in situ released by cells) based on the combination of mussel-inspired biomimetic adhesion technique, 3D micro/nano-biointerface construction and electrochemical biosensing technique.

1. Introduction

Superoxide anions ($O_2^{\bullet -}$), as a typical type of reactive oxygen species (ROS), is produced by organism deviating from the usual standards, which may lead to a higher incident rate of cancer risk, an accelerated aging and some forms of neural degeneration with irreversible consequences such as Parkinson's disease [1–5]. Therefore, it is of vital importance to find a suitable method that can detect $O_2^{\bullet -}$ reliably, promptly, and explicitly, which is supposed to be easy to implement and suitable for routine diagnosis, pathological analysis, and operation for daily physical examination.

Among the various methods developed for detection $O_2^{\bullet -}$ thus far [6–8], electrochemical method, with the advantages of fast response, simple operation and high sensitivity [9–14], has been received wide attention. Amperometric enzyme electrodes for $O_2^{\bullet -}$ have been developed with various electrode materials, such as cytochrome c and superoxide dismutase (SOD) [15–17]. However, enzyme-based biosensors were difficult to be used widely owing to the inherent weaknesses of the enzyme, such as high volatility, high cost, and difficulty in storage. One of the most promising materials is manganese (Mn) compound as the biomimetic enzyme that takes advantage of catalytic dismutation of $O_2^{\bullet -}$ [18]. Silica-manganese phosphate (SiO_2-$Mn_3(PO_4)_2$) nanoparticles were synthesized by surface self-assembly processes for $O_2^{\bullet -}$ detection as reported in our previous work [19].

Until now, most of cytosensors for detection of cellular molecules in situ were based on a two dimensional (2D) detected platform that utilized to capture the detected cells. In fact, real cells inhabit a three dimensional (3D) space with complicated microenvironment [20–23]. So if a simulating real 3D environment can be offered for the detected cells, it will facilitate the authenticity and accuracy of cell electrochemical detection. 3D nickel foam has attracted increasing attentions due to its unique 3D scaffold structure, low cost, good magnetic and conductivity [24,25].

In this work, 3D nickel foam was introduced to create a simulating real 3D environment for rapid and accurate detection of $O_2^{\bullet -}$ from detected cells. Firstly, magnetic manganese phosphate nanoparticles

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(Fe_3O_4-$Mn_3(PO_4)_2$ NPs) synthesized by surface self-assembly technology were directly and robustly anchored on 3D nickel foam based on the mussel-inspired adhesion technique. Then, this 3D nickel foam was attracted by magnetic electrode to construct a novel biosensor with 3D interface for $O_2^{\bullet -}$ detection. The as-obtained biosensor, based on a 3D micro/nano-biointerface as an amicable platform to promote cells adhesion, could not only greatly shorten detection cycle and simplify test process, but also shorten the diffusion distance between $O_2^{\bullet -}$ released from cell and the medium solution for enhanced sensing performance. This biosensor provided a new opportunity for the design and application of 3D electrode materials with enhanced electrocatalytic performance in biosensing.

2. Experimental section

2.1. Materials

Ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$) and tetraethyl orthosilicate (TEOS) were purchased from Sinopharm Chemical Reagent Co. Ltd. Sodium acetate (NaAc) was obtained from Shanghai Lingfeng Chemical Reagent Co. Ltd. (3-Aminopropyl) triethoxysilane (APTES), phytic acid (PA, 70 wt%) solution and phytic acid sodium salt hydrate were purchased from Aladdin Chemistry Co. Ltd (Shanghai, China). Manganese sulfate monohydrate ($MnSO_4{\cdot}H_2O$), ethylene glycol (EG), dimethyl sulfoxide (DMSO) were obtained from Sinopharm Chemical Reagent Co. Ltd. Potassium hyperoxide (KO_2) was purchased from Alfa Aesar. Triton X-100, 18-crown-6, phorbol 12-myristate 13-acetate (PMA), dopamine hydrochloride (DA), cysteine (Cys), ascorbic acid (AA) and uric acid (UA) were acquired from Aladdin Sigma-Aldrich Co. (USA). Hydrogen peroxide (H_2O_2, 30%) was purchased from Beijing Chemical Works (Beijing, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), and penicillin/streptomycin (P/S), 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Nanjing Heyao Biotech. Co., Ltd. (Nanjing, China). Ni foam was purchased from Wuzhou Sanhe New Mater Co., Ltd. (Guangxi, China).

2.2. Preparation of Fe_3O_4 NPs

The Fe_3O_4 NPs were synthesized in accordance with the procedure described in the literature and functionalized via solvothermal route [26–29]. 4.04 g $FeCl_3{\cdot}6H_2O$ was dissolved in 100 mL EG to form a clear solution in a three-necked flask, then 8.20 g NaAc was added quickly under constant and vigorous stirring for 0.5 h. The obtained solution was subsequently moved to a Teflon-lined autoclave (50 mL) that was sealed for 8 h with heating to 200 °C, and then naturally cooled down to room temperature. The resulting black precipitate was got via centrifugation, washed repeatedly with distilled water and absolute ethanol, and dried at 50 °C inside a vacuum oven for 5 h.

2.3. Preparation of Fe_3O_4-$Mn_3(PO_3)_2$ NPs

Fe_3O_4-$Mn_3(PO_3)_2$ NPs fabricated with layer-by-layer (LBL) self-assembly strategy [30–32] exhibited size-controlled (Scheme 1A). First, 0.10 g of Fe_3O_4 NPs were ultrasonically treated with 50 mL, 0.1 M HCl aqueous solution for 10 min. Afterwards, the magnetite particles were isolated, and washed with deioned water, and then homogeneously dispersed in a mixture solution (80 mL ethanol, 20 mL deioned water, and 1.0 mL, 28 wt% ammonia), to which TEOS (0.144 mM) was subsequently added. After the mixture had been stirred at room temperature for 6 h, an appropriate amount of APTES was added to it to form the Fe_3O_4-NH_2 NPs solution in Scheme 1A (a). After the solution had been stirred for 30 min, 120 μL of PA/PA sodium salt hydrate buffer solution (pH = 7) was injected into in under incessant stirring for 24 h. The resulting NPs were washed with alcohol and double-distilled water (Scheme 1A(b)).

Finally, the Fe_3O_4-PA NPs were redispersed in 10 mL of water, and then 10 mL of $MnSO_4$ solution (12 mM) were injected to the round-bottomed flask containing Fe_3O_4-PA NPs under constant stirring for 1 h at 60 °C. At the end of the reaction, the obtained materials were collected via centrifugation, washed with double-distilled water, and dried in a vacuum oven at 60 °C for 24 h (Scheme 1A(c)).

2.4. Preparation of Fe_3O_4-$Mn_3(PO_3)_2$@Ni foam

Fe_3O_4-$Mn_3(PO_3)_2$ NPs were anchored onto Ni foam in an aqueous solution of dopamine in Scheme 1B (a), which was inspired by the bioadhesion of marine mussels [33,34]. Briefly, 100 mg of Fe_3O_4-$Mn_3(PO_3)_2$ NPs and 40 mg of dopamine hydrochloride were dispersed in 20 mL of H_2O at pH = 9 by ultrasonication. The pH of the dispersions was adjusted by NaOH. Then a piece of Ni foam was immersed in the above dispersions under stirring for 12 h. After it was rinsed with water and dried at 80 ℃, the foam covered with as-papered samples was obtained a 3D micro/nano-biointerfaces for further cells adhesion (Scheme 1B(b)).

2.5. Preparation of superoxide anion

Dissolving KO_2 to DMSO which contained 18-crown-6, a stabilized $O_2^{\bullet -}$ solution was attained. According to the molar absorptivity of $O_2^{\bullet -}$ in DMSO, the concentration of $O_2^{\bullet -}$ was supervised by minuting the deoxidation at 550 nm using ferricytochrome c spectrophotometrically [35].

2.6. Culture of cells

The A549 cells were grown at 37 °C in 5% CO_2 culture medium with DMEM, 10% fetal bovine serum and 1% P/S. The pretreated cells, gathered by centrifugation, was added into the PBS solution including 100 μg mL^{-1} PMA and 50 mM glucose.

2.7. MTT assays

The viable cells viabilities of the Fe_3O_4-$Mn_3(PO_4)_2$@Ni foam for different incubation time were appraised using MTT assays. A549 Cells, at a density of 0.5×10^5 cells, and synthesized Fe_3O_4-$Mn_3(PO_4)_2$@Ni foams were seeded in a 96-well plate for 4 h, 12 h, 24 h incubation period. After then, the wells were treated with 50 μL MTT (2 mg mL^{-1}) and incubated for another 4 h at 37 °C. The medium was then displaced with 150 μL of DMSO, and a microplate reader was carried out by monitoring the absorbance at a 570 nm.

2.8. Cell capture experiments

1 mL of 5×10^4 cells suspension was seeded onto a 24-well plate with the different samples. After 60 min incubation time at 37 ℃, the samples were rinsed with PBS for three times. The captured cells on the substrates were fixed with 2.5% GA in PBS for 4 h. The substrate was subsequently dehydrated with in a series of ethanol gradient solution with different volume fractions (30%, 50%, 70%, 90% and 100%) at each concentration for 20 min, and finally dried at room temperature to observe the morphology of the cells on the samples by SEM.

After 4 h fixed treatment with 2.5% GA, the films were covered with a DAPI solution (2 μg mL^{-1}) for 15 min, followed by PBS washed. At last, the samples were kept at 4 ℃ in dark for the subsequent fluorescence microscope.

2.9. Apparatus and electrochemical measurements

The morphologies of the as-prepared samples were detected by TEM and high-resolution transmission electron microscopy (HITACHI H-7650, Japan). FTIR of the sample were performed from a VARIAN Cary