



Quantum dots-hyperbranched polyether hybrid nanospheres towards delivery and real-time detection of nitric oxide



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ABSTRACT

In this work, novel hybrid nanosphere vehicles were synthesized for nitric oxide (NO) donating and real-time detection. The hybrid nanosphere vehicles consist of cadmium selenide quantum dots (CdSe QDs) as NO fluorescent probes, and the modified hyperbranched polyether (mHP)-based diazeniumdiolates as NO donors, respectively. The nanospheres have spherical outline with dimension of ~127 nm. The data of systematic characterization demonstrated that the mHP-based hybrid nanosphere vehicles (QDs-mHP-NO) can release and real-time detect NO with the low limit of 25 nM, based on fluorescence quenching mechanism. The low cell-toxicity of QDs-mHP-NO nanospheres was verified by means of MTT assay on L929 cells viability. The QDs-mHP-NO nanospheres provide perspectives for designing a new class of biocompatible NO donating and imaging systems.

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1. Introduction

Nitric oxide (NO) is a free radical molecule that can be served as a multifaceted signaling and bio-regulatory molecule in mediating multiple biological events; it is generally generated in various tissues from the decomposition of amino acid *L*-arginine by different forms of nitric oxide synthase [1]. NO always plays a very important effect on regulating the cardiovascular system, the central and peripheral nervous systems, and the immune system [2–8]. Various compounds that can store NO attracted great interest, because of their potential to provide NO in a wide variety of conditions or applications. Diazeniumdiolates are certain compounds containing the anionic $[N(O)NO]^-$ functional group, which is typically synthesized by reactions of a nucleophile with NO at elevated pressure [9,10] and could decompose spontaneously to generate NO under physiological conditions (37 °C, pH = 7.4).

In recent years, semiconductor quantum dots (QDs) have obtained great attention because of their unique properties and advantages over conventional organic fluorophores, such as broad excitation spectra, excellent photochemical stability, and narrow, symmetric and tunable emission spectra as a result of quantum confinement effect [11–15]. QDs had been widely used in biolabeling and biosensing [16–20]. It also could be adopted as a new class of fluorescent probes for NO sensing

[21–23], because of the fluorescence changes of the QDs caused by the interaction between NO and QDs [24,25].

During the last decades, hyperbranched polymers have attracted considerable attention because of their unique molecular characteristics and properties, such as highly branched and spherical three-dimensional molecular structure, large amounts of end groups, little chain entanglement, high solubility and low melt and solution viscosity [26–28]. Various polymerization methods have been employed to produce hyperbranched polymer [29–33]. With chemically modifiable functional groups on their terminal branching units, hyperbranched polymers have become suitable nanocarriers for bioactive agents [34]. Especially, hyperbranched polymers have been used to design nanoscale delivery systems for drugs [35–38]. Hyperbranched polymeric drug carriers provide advantages over small-molecular-weight drug carriers in that they possess plenty of binding entities and are highly efficient in drug loading and delivery. Currently, most of the hyperbranched polymeric drug carriers are loaded with solid-state drugs. Those designed to deliver gaseous drugs for therapeutical purposes are rarely reported.

Diazeniumdiolates exhibit high efficiency in NO generation, yet they have an obvious drawback wherein the release of NO from them is difficult to control. Therefore, incorporating fluorescent probes for NO into diazeniumdiolate systems to detect and monitor the NO release in real time is of great importance. Tan et al. have reported the chitosan based QDs nanocomposite NO donors capable of releasing NO and simultaneously detecting the NO release [39,40]. However, the reaction between the molecule and NO was so rapid that the detection capacity tended to be saturated before the NO release finished,

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and it is difficult to find a linear relationship between the fluorescence increase and the released amount of NO. In our previous study, we find that the hyperbranched polyether (mHP) nanospheres can deliver and selectively detect NO against oxidation products from NO [41].

In the present work, the NO delivery vehicle consisting of modified hyperbranched polyether (mHP) nanospheres with fluorescent cadmium selenide (CdSe) QDs encapsulated in them was synthesized. Systematic characterization of QDs-mHP-NO nanospheres, i.e., the structure and morphology, the fluorescence properties, and the NO releasing capability was well performed. Moreover, the cytotoxicity, in vitro NO release, real-time fluorescence imaging and the NO detection capability were also fully investigated.

2. Materials and methods

2.1. Materials

Boron trifluoride etherate (BF₃OEt₂) and 3-ethyl-3-oxetanemethanol (EOX) were provided by Sigma-Aldrich. *N*-(3-(Trimethoxysilyl)propyl) ethylenediamine (NTPED) and diamine tetraacetic acid (EDTA) were provided by Aladdin Reagent, Ltd. Ethylene oxide, mercaptoethylamine, ethylene dichloride, cadmium acetate, selenium (≥99.9%), sodium borohydride, ethyl bromide, dimethyl formamide (DMF), dimethyl sulfoxide (DMSO), sodium methoxide, toluene and methonal were all purchased from Sinopharm Chemical Reagent Co., Ltd. Nitric oxide gas (NO, 99.9%) was purchased from Foshan Kodi Gas Chemical Industry, China. NO solutions of varied concentrations used for investigating the sensitivity of the QDs-mHP-NO nanospheres to NO were prepared by making a series of dilutions of a saturated NO solution (≈1.8 mM) [1]. L929 cells and fetal bovine serum (FBS) were kindly provided by the Institute of Biochemistry and Cell Biology, CAS. Phosphate buffered saline (PBS) buffers were prepared in our own lab. All the chemicals were of analytical grade unless otherwise stated. The water used was deionized water.

2.2. Synthesis of modified hyperbranched polyether (mHP) nanospheres

Firstly hyperbranched polyethers (HP) were prepared by cationic ring-opening polymerization, which is similar to the method reported previously [42]. The solvent ethylene dichloride (560 mL) and the catalyst BF₃OEt₂ (49.7 g, 0.35 mol) were added into a three-necked flask that had been purged with nitrogen gas for 30 min. Then the monomer EOX (0.7 mol, 81.2 mL) was gradually introduced into the flask within 5 min, stirred vigorously. The reaction was allowed for 48 h at different temperatures, and then terminated with ethanol. Subsequently, the temperature of the system was decreased to 0 °C, and a certain amount of ethylene oxide was added. After 24 h, the residual solvents and catalyst were removed under reduced pressure. The resultant product (3.5 g) was placed in a three-necked flask, followed by addition of 3.5 mL NTPED and 350 mL toluene. The mixture was refluxed at 80 °C under argon protection for 10 h, and then centrifuged and washed by toluene to remove the unreacted NTPED. The products were dried under vacuum at 60 °C to obtain mHP nanospheres.

2.3. Preparation of mHP-NO nanospheres

The mHP nanospheres were suspended in a mixture of MeOH and NaOMe at a molar ratio of [Na⁺]/[NH] = 3. The high-pressure reactor was first flushed with nitrogen and then degassed under vacuum. Fresh NO gas was introduced into the reactor at the pressure of 100 psi for 5 days. After the reaction was complete, the reactor was again flushed with nitrogen gas. The products were filtered, washed with ether, and dried at room temperature under vacuum. The NO

loading efficiency of the mHP-NO nanospheres was 34.3%, estimated based on the following equation:

$$\text{NO loading efficiency \%} = \frac{\text{released NO } (\mu\text{ mol})}{\text{amount of NTPED } (\mu\text{ mol})} \times 2 \times 100 \% \quad (1)$$

2.4. Synthesis of CdSe QDs

The CdSe QDs were synthesized according to the procedure reported by Tan et al. [24]. Specifically, 79 mg of selenium powder and 16 mg of sodium borohydride were dissolved in deionized water in a reaction flask. The reaction mixture was stirred vigorously at 30 °C until it became colorless. 0.367 g of cadmium chloride was dissolved in water in a three neck flask under nitrogen flow. 0.38 mL of mercaptoethylamine was then added into the cadmium chloride solution. Subsequently, the as-prepared sodium hydrogen selenide solution was rapidly injected into the above mixture, whose pH value was adjusted to 9 by adding 1 M sodium hydroxide solution in it. The mixture was allowed to react under stirring at 100 °C for 3 h. The resultant reddish brown colloidal solution was dialyzed against water for 48 h to remove excess surfactants and unreacted ions. Finally it was concentrated on a rotary evaporator and centrifuged at 8000 rpm/min to obtain CdSe QDs.

2.5. Preparation of QDs-mHP and QDs-mHP-NO nanospheres

3.5 g of mHP-NO nanospheres were dispersed in water at room temperature, followed by addition of 10 g of EDTA under stirring. The as-synthesized CdSe QDs were then added into the above solution, stirred for 3 min. Subsequently, ethanol was added dropwise to the solution, stirred until the solution turned cloudy. The resultant colloidal solution was filtered, centrifuged, and the sediment was dialyzed against deionized water for 12 h to remove EDTA. After thoroughly washing with ether and drying under vacuum, yellow QDs-mHP-NO nanospheres were obtained. The NO loading efficiency of the nanospheres calculated according to Eq. (1) was 29.5%, lower than that of the mHP-NO nanospheres, presumably because some diazeniumdiolate groups have decomposed during the conjugation of QDs. The QDs-mHP nanospheres were prepared with the same method as that for the QDs-mHP-NO nanospheres.

2.6. Characterization

Transmission electron micrographs (TEM) were recorded on a JEM-2100 transmission electron microscope (JEOL, Japan) at 200 kV. Samples were suspended in ethanol, fully dispersed by ultrasonic wave, and deposited on a 300 mesh copper grid prior to observation. The size distribution of the QDs-mHP-NO nanospheres was determined by a Nano ZS90 particle size and zeta potential analyzer (Malvern, UK) based on dynamic light scattering (DLS) at a scattering angle of 90°. The weight-average molecular weight of HP was measured on a HLC-8320GPC gel permeation chromatography (TOSOH, Japan) using DMF as the eluate. Nuclear magnetic resonance (NMR) tests were carried out on a Varian Mercury Plus-400 spectrometer at 400 MHz. Fourier transform infrared spectroscopy (FTIR) tests were conducted on a Spectrum 100 FTIR spectrometer (PerkinElmer, US). Samples were dried, powdered, and made into transparent films by mixing them with KBr. Surface analysis was conducted on an ESCALAB 250 X-ray photoelectron spectrometer (XPS, Thermo Scientific, US) with non-monochromatic Al K_α X-ray (1486.6 eV). The analyzer was operated at 20 eV pass energy with an energy step size of 1 eV. Binding energy calibration was based on C 1s at 284.6 eV. UV absorption spectrum was recorded in the 200–350 nm range using a Lambda 35 UV-Vis spectrophotometer (PerkinElmer, US). The fluorescence spectra of the samples were

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