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Novel quantum dots–carboxymethyl chitosan nanocomposite nitric oxide donors capable of detecting release of nitric oxide in situ

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ABSTRACT

Nitric oxide (NO) donor compounds are primarily monofunctional in that they release NO under the requisite conditions. To detect the amount and duration of NO released, subsequent analysis methods are required. It would be advantageous if a NO donor compound could both release and detect NO at the same time. This would eliminate potential errors in the analysis. In this paper, novel cadmium telluride quantum dots (CdTe QD)–carboxymethyl chitosan (CMCS) nanocomposite NO donors, including both diazeniumdiolates and fluorescence probes, were fabricated by first synthesizing CdTe QD in CMCS aqueous solution and then reacting NO as well as ethyl bromide with the resultant CdTe QD–CMCS nanocomposites. Transmission electron microscopy, scanning electron microscopy and particle size analysis were used to examine the morphology and size distribution of the CdTe QD–CMCS nanocomposite NO donors. The donors are nanospheres with CdTe QD encapsulated and have dimensions of ~300 nm. Fourier transform infrared spectroscopy, X-ray diffraction, X-ray photoelectron spectroscopy and contact angle tests were employed to characterize the chemical structure of the donors, and the results also show that CdTe QD are well incorporated into CMCS, and many of them are close to the surface of the donors. The precursors of the donors exhibit a fluorescent effect, and the fluorescence can be quenched by NO. The donors can release NO spontaneously in a phosphate-buffered saline solution similar to a physiological environment, and can quantitatively detect the release of NO in situ based on fluorescence quenching of the donors by the NO.

1. Introduction

Nitric oxide (NO), a free radical molecule possessing very important functions in biological systems, is generated in various tissues from the amino acid L-arginine by different forms of NO synthase [1]. Many NO donors have been designed and synthesized, among which diazeniumdiolates are of great interest [2,3]. Diazeniumdiolates represent a class of compounds containing the anionic $[N(O)NO]^-$ functional groups, typically synthesized by reactions of a nucleophile with NO at elevated pressure [4]. They can generate NO under physiological conditions at different rates. Some diazeniumdiolate carriers, however, are found to be toxic and harmful to the human body, which limits their application in treatment of relevant diseases. Chitosan is a natural polysaccharide composed of β-(1 → 4)-2-amido-2-deoxy-D-glucan (glucosamine) and β-(1 → 4)-2-acetamido-2-deoxy-D-glucan (acetyl glucosamine) units, produced by deacetylation of chitin extracted from the exoskeleton of crustaceans [5]. Chitosan and its derivatives are biodegradable and biocompatible cationic polymers, which have been widely used in biomedical areas [6]. They have excellent chelating and adsorption characteristics owing to the high hydrophilicity and activity of hydroxyl and amino groups, which can react with metal cations and uptake metal cations by chelation mechanism. Also, the flexible structure of the polymer chains enables them to adopt suitable configuration for complexation with metal ions [7,8]. The secondary amine groups in some chitosan derivatives can act as a nucleophile to which NO is attached. The resultant chitosan–NO adduct has the $[N(O)NO]^-$ groups and is capable of releasing NO sustainably [9]. This polymeric diazeniumdiolate has proved an effective and reliable source of NO in a physiological environment.

Semiconductor quantum dots (QD) have 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 [10–15]. QD are usually prepared in aqueous solutions under alkaline conditions [16,17]. As a new class of fluorescent probes, QD are currently under intensive study in biosensing and biolabeling [18–22]. To reduce biological toxicity as well as enhance water solubility, QD are often modified by a variety of small molecules and biomacromolecules. Chitosan or chitosan

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derivatives-modified QD exhibited long-term fluorescence stability and excellent biocompatibility [15,23] and are promising drug release carriers. QD are also attractive for their fluorescence efficiency and sensitivity to the presence and nature of attached molecules, ions and/or adsorbates at their surface. Sensing of NO by QD based on photoluminescence (PL) has been reported [24–26]. Water-soluble QD can be used for the analysis of NO in aqueous solutions under physiological conditions.

Although there have been reports of chitosan- or chitosan derivatives-based NO donors, detecting the release of NO cannot be achieved unless independent fluorescence probes are introduced together with the donors. In the present work, a novel system is designed, i.e., cadmium telluride quantum dots (CdTe QD)–carboxymethyl chitosan (CMCS) nanocomposite NO donors, including both diazeniumdiolates and fluorescence probes. CMCS was selected as the supporting material because it is water soluble and has a great number of secondary amine groups that can react with NO to achieve high NO loading. This new system can release NO spontaneously in a physiological environment, and can meanwhile detect the release of NO based on fluorescence quenching of the donor by NO. The chemical composition and microstructure of the CdTe QD–CMCS nanocomposite NO donors were examined. The NO releasing and detection properties were investigated, and cell tests were also carried out to study the feasibility of the CdTe QD–CMCS nanocomposite NO donors for biomedical applications.

2. Materials and methods

2.1. Materials

CMCS (weight-average molecular weight M_w = 400 kDa, degree of deacetylation 90%, degree of carboxymethylation 1.112) was obtained from chitosan provided by Sinopharm Chemical Reagent Co. (China). Cadmium acetate (A.R.), tellurium ($\geqslant$99.9%), sodium borohydride (A.R.), ethyl bromide (A.R.) and dimethyl formamide (DMF, A.R.) were all purchased from Sinopharm Chemical Reagent Co. Nitric oxide gas (NO, 99.9%) was purchased from Foshan Kodi Gas Chemical Industry (China). Saturated NO solutions ($\sim$1.8 mmol L^{-1}) were prepared according to the reported standard procedure [1]. NO standard solutions were prepared by making a series of dilutions of a saturated NO solution. NO solutions were made fresh and were kept in a brown glass flask with a rubber septum. Porcine iliac artery endothelial cells (PIEC) and bovine serum albumin (BSA) were kindly provided by the Institute of Biochemistry and Cell Biology, CAS. Phosphate-buffered saline (PBS) solutions (0.01 M and 0.2 M, pH = 7.4) were prepared in the authors' own lab.

2.2. Preparation of CdTe QD–CMCS

Sodium hydrogen telluride was obtained via reaction between sodium borohydride and tellurium in water. Under magnetic stirring, 0.5 g CMCS was dissolved in 250 ml cadmium acetate (0.046 g) aqueous solution to facilitate a chelation balance at pH $\approx$ 8 (adjusted by 0.1 M sodium hydroxide aqueous solution), followed by dropwise addition of 10 ml aqueous solution of sodium hydrogen telluride (0.031 g) under vigorous stirring at room temperature. The mixture was purged with high-purity nitrogen gas for 20 min and subsequently refluxed under stirring at 95 °C for 3 h. The resultant colloidal solution was dialyzed against water to remove unreacted molecules and ions. Then it was concentrated on a rotary evaporator and eventually freeze-dried to yield yellow CdTe QD–CMCS. The dried CdTe QD–CMCS was weighed and then analyzed by thermogravimetry (TG), which was performed on a Q5000IR thermogravimetric analyzer (TA, US) under N_2 flow in the temperature range 25–800 °C at a heating rate of 20 °C min^{-1}. The loading of QD, determined from the TG results, was 8 wt.%. The PL quantum yield of the CdTe QD–CMCS was 47%, with quinine sulfate as the reference fluorochrome.

2.3. Synthesis of CdTe QD–CMCS diazeniumdiolates and CMCS diazeniumdiolates

The CdTe QD–CMCS or CMCS was 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 100 psi for 7 days. On completion of the reaction, the reactor was again flushed with nitrogen gas. The products were filtered, washed with ether and dried at room temperature under vacuum. They were stored in an airtight container placed in a dessicator at 20 °C before use.

2.4. Preparation of CdTe QD–CMCS nanocomposite NO donors

As diazeniumdiolate ions are typically heat and acid sensitive, they are often protected during manipulations [27]. O^2-substituted diazeniumdiolates, which have enhanced stability, can be produced by reacting electrophiles (aryl halides, alkane) with the diazeniumdiolate anions [28,29]. In the present work, ethyl bromide was used to provide a protecting group for the diazeniumdiolates. The CdTe QD–CMCS diazeniumdiolates were suspended in DMF in a sealed flask, kept at 0 °C and stirred under a nitrogen atmosphere. Ethyl bromide was injected into the flask, and reaction was allowed for 3 h. The mixture was then heated to room temperature and stirred for another 48 h. The products were filtered, washed by diethyl ether three times, and dried for 24 h in a vacuum drying oven to obtain CdTe QD–CMCS nanocomposite NO donors, whose PL quantum yield was 41%.

2.5. Cell culture

PIEC were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10 wt.% BSA in a humidified incubator with 5 vol.% carbon dioxide at 37 °C. The medium was refreshed every other day. The PIEC were rinsed with a 0.01 M PBS solution containing 10 wt.% BSA before observation.

2.6. Cell viability tests

The cytotoxicity of the CdTe QD–CMCS nanocomposite NO donors was evaluated by 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium chloride (MTT) viability assay. The PIEC were seeded in 96-well culture plates at a density of 4000 cells per well and incubated at 37 °C for 24 h for cell attachment. The culture medium in each well was then replaced by a fresh medium containing CdTe QD–CMCS NO donors at different concentrations (0.1–1 mg ml^{-1}). One row of the 96-well plates was used as the control. After incubation for 24 h, the cells were treated with MTT reagent for 4 h to form purple colored formazan. After the formazan had been dissolved in dimethyl sulfoxide, the absorbance of individual wells was recorded at 570 nm, using a Multiskan MK3 Enzyme-labeled Instrument (Thermo Scientific, US). Triplicates were set up for each sample concentration.

2.7. Characterization

Transmission electron microscopy (TEM) images were recorded on a JEM-2100 transmission electron microscope (JEOL, Japan) at 200 kV. Samples were suspended in ethanol, fully dispersed by