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Nitrogen and phosphorus co-doped graphene quantum dots as a nano-sensor for highly sensitive and selective imaging detection of nitrite in live cell

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ABSTRACT

Doping graphene quantum dots (GQDs) with heteroatoms can provide new or improved structural and optical properties. Therefore, heteroatom doped GQDs have promising applications in environmental and biological fields. In this work, we present a novel nitrogen and phosphorus co-doped graphene quantum dots (N,P-GQDs), which were directly prepared *via* a facile hydrothermal method using tetrakis (hydroxymethyl) phosphonium chloride and ethylenediamine endcapped polyethylenimine as precursors. N,P-GQDs with two different doping ratios of nitrogen and phosphorus were prepared at different temperatures (230 and 250 °C). This N,P-GQDs showed good dispersity, thickness of 1–3 atomic layers and sizes of less than 20 nm. The N,P-GQDs were water dispersible, highly luminescent (with an absolute quantum yield of 9.4%), and showed excitation and concentration-dependent behavior. Moreover, the N,P-GQDs exhibited a sensitive response to NO_2^- in the concentration range of 5–30 nM with the detection limit of 2.5 nM. Significantly, cell toxicity experiment showed that the N,P-GQDs had negligible cytotoxicity and excellent biocompatibility. Thus, the N,P-GQDs could serve as a powerful fluorescent nano-sensor for imaging detection of nitrite in live cell.

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1. Introduction

In recent years, photoluminescent carbon dots (C-dots) or graphene quantum dots (GQDs) have attracted increasing attention owing to their chemical inertness, low cytotoxicity, excellent biocompatibility, good dispersibility in water, and stable photoluminescence (PL). This makes them most promising fluorescent nanomaterials [1–4]. However, the photoluminescence quantum yields of GQDs are extremely low, ranging from 1 to 5%, indicating that pristine GQDs have a high density of defect states [5,6]. Fortunately, later studies have shown that the quantum yields of GQDs can be improved by using heteroatom doping, such as nitrogen [7], boron [8], sulfur [9], phosphorus [10], and fluorin [11]. Doping with different heteroatoms may induce some changes in GQDs composition, morphology and structure, presenting distinctive properties. Notably, the Lee's group has synthesized near-infrared photoluminescent graphene quantum dots with zigzag edges by using strong acid treatment and nitrogen doping [12]. Dong et al. reported the synthesis of nitrogen and sulfur co-doped carbon-based dots (N,S-CDs) with a high photoluminescence quantum yield (73%) through a one-step hydrothermal treatment [13]. On the other hand, heteroatom doping can also change the sensitivity of GQDs to their environment. Thus, doped GQDs can be used as an ultrasensitive fluorescent probe for the detection of different analytes, such as Fe^{3+} [14], Ag^+ [15], chloramphenicol (CAP) [16], and 2,4,6-trinitrophenol (TNP) [17].

Nitrite (NO_2^-) is an important inorganic anion that is usually used as a food additive or a preservative agent [18]. However, it is also considered to be a cancerogen owing to its ability to interact with amines and proteins to generate highly carcinogenic N-nitrosamines, which enhance the possibility of cancer and deformities [19]. Contamination of rural drinking water supplies with nitrite by livestock waste, organic wastes, and chemical fertilizers continues to be a problem throughout the world [20]. In addition, nitrite is also an important metabolite in many physiological and pathological processes [19]. Monitoring nitrite in living cells is of great benefit to understand the mechanism of cell apoptosis.

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Therefore, the rapid, low-cost, and sensitive detection of nitrite ion is of great importance in environmental and biological systems. Previously reported methods for the detection of nitrite include ion selective electrode [21], chemiluminescence [22], and electrochemical techniques [23]. However, these methods usually involve complicated or time-consuming processes, expensive equipment, and high costs, whereas fluorescence analysis is currently considered to be a highly effective method of nitrite detection. So far, only a few fluorescent probes such as organic probe [24], Cu nanoclusters [25], dimer [26], and carbon nanoparticles [27] have been reported to determine the nitrite. Notably, detection of nitrite in live cell is still challenging due to the lack of highly selective, highly sensitive, simple and rapid methods. Thus, synthesis of highly selective and sensitive fluorescent nano-sensor for imaging detection of nitrite in live cell is expectant.

In this work, a novel nitrogen and phosphorus co-doped GQDs (N,P-GQDs) were synthesized through a hydrothermal carbonization method by using tetrakis(hydroxymethyl) phosphonium chloride (THPC) and ethylenediamine endcapped polyethylenimine (PEI-EC) as precursors (Fig. 1a). THPC served as the carbon and phosphorus source, while the PEI-EC with NH_2 groups provided nitrogen source and as a passivation agent. The obtained N,P-GQDs had sizes of less than 20 nm and not only exhibited high crystallinity with thickness of 0.36–0.65 nm, and lower cytotoxicity, but also presented superior optical properties, such as strong PL, tunable full-color emission, and high photo-stability. Thus, the N,P-GQDs were highly suitable for live cell labeling and imaging. Furthermore, we demonstrated that the N,P-GQDs can be utilized as fluorescent nano-sensor for imaging detection of nitrite in live cell.

2. Experimental

2.1. Reagents and materials

THPC and PEI-EC were purchased from Sigma Aldrich. Other chemicals are analytically pure and used as received without further purification. Water was purified by employing a Milli-Q plus 185 equip from Millipore (Bedford, MA) and used throughout the work.

2.2. Synthesis of N,P-GQDs

A mixed solution was prepared by dissolving 1.5 mL of THPC and 500 μL of PEI-EC in 25 mL of water. Then, the mixed solution was transferred into a 50 mL Teflon equipped stainless steel autoclave and placed in a drying oven at 230 °C or 250 °C for 8 h. After cooling to room temperature, the yellow liquid (acidic solution) was adjusted to 7.0 by adding NaOH solution under stirring. The obtained mixture was dialyzed in a dialysis bag for 24 h (molecular-weight cutoff of 1000) to remove small molecular substances. Then the obtained N,P-GQD was preserved for further characterization and use.

2.3. Characterization of N,P-GQDs

The UV–vis absorption spectra of N,P-GQDs were recorded on a Cary 60 UV–vis spectrophotometer (Agilent Technologies, Australia). All fluorescence spectra were obtained on a Cary Eclipse fluorescence spectrometer (Agilent Technologies, Australia). Fluorescence lifetime experiments were performed by a FL3-P-TCSPC time-resolved fluorescence spectrometer (Horiba Jobin Jvon). The absolute quantum yields were measured on FLS980 Edinburgh Fluorescence Spectrometer (Edinburgh, UK) with an integrating sphere. Transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HRTEM) images of N,P-GQDs were operated at a voltage of 300 kV on an FEI Tecnai G2 F-30 field emission HRTEM (Holand). Atomic force microscopy (AFM) images were obtained by a Multimode Nanoscope IIIa controllor AFM (USA). The X-ray diffraction (XRD) pattern of N,P-GQDs was measured using a diffractometer (Rigaku D/max 2500, Japan) with Cu Ka radiation. X-ray photoelectron spectroscopy (XPS) datas were obtained with a Thermo ESCALAB 250 × I electron spectrometer from VG Scientific using 150 W Al Ka radiation. FT-IR spectrum of the N,P-GQDs was recorded on FT-IR (PerkinElmer, USA) spectrometer. The Raman spectra of N,P-GQDs were measured on Laser Micro-Raman spectrometer (Invia, Renishaw, England).

2.4. Detection of NO_2^-

A 20 μL volume of the N,P-GQDs solution was mixed with 60 μL Tris-HCl buffer solution (10 mM pH 7.0). After that, 20 μL of different concentrations of nitrite solution was added with gentle shaking. The fluorescence spectra were recorded 20 min later within the wavelength range of 360 nm to 600 nm at 350 nm excitation.

2.5. MTT assay

Human bladder carcinoma T24 cells were harvested (the cell density was adjusted to 10^5 cells per mL) and seeded in a 96-well plate overnight for 24 h. Then, the N,P-GQDs solution was added into each well with increasing concentrations from 20 to 100 μg/mL. After that, the cells were cultivated for 24 h, and 10 μL of 1 mg/mL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution was added to each cell well. After incubation for 4 h, the culture medium was discarded, and 100 μL of dimethyl sulfoxide was added. The obtained mixture was shaken for 15 min in the dark at room temperature, and the absorbance of each well was measured at 570 nm using an enzyme linked immunosorbent assay (ELISA) reader with pure DMSO as a blank. A non-treated cell was used as a control and the relative cell viability (mean% ± SD, n = 3) was expressed as Abssample/Abscontrol × 100%.

2.6. Live cell imaging

Human bladder carcinoma T24 cells were maintained in Dulbecco's modified eagle medium (DMEM) supplemented with FBS (10%) and 100 mg/mL streptomycin and 100 units/mL penicillin solution in 5% CO_2 at 37 °C. Cells were grown on the bottom of 35 mm glass culture dishes to the density of 70% confluence and then incubated with N,P-GQDs at 37 °C for 20 h. Afterwards, the cells were washed three times with PBS (pH 7.4). Images of the cells were immediately captured at ambient temperature on a laser scanning confocal microscopy (Zeiss LSM710, Germany) using a digital camera.

3. Results and discussion

3.1. Characterization of the N,P-GQDs

The N,P-$GQDs_{230\,°C}$ and the N,P-$GQDs_{250\,°C}$, which were respectively heated at a reaction temperature of 230 °C and 250 °C, were synthesized *via* hydrothermal method. The morphology and structure of the as-prepared N,P-GQDs were characterized using transmission electron microscopy (TEM), high-resolution transmission electron microscopy (HRTEM), and atomic force microscopy (AFM). Fig. 1b shows the TEM image of the N,P-$GQDs_{230\,°C}$, which is well-dispersed in water with a size distribution in 1.5–7.5 nm range and an average size of 4.2 nm (shown in the inset). Fig. 1c is the inverse fast Fourier transform (IFFT)

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