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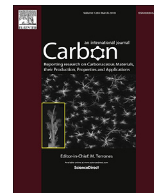
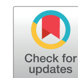


Carbon dots with red-shifted photoluminescence by fluorine doping for optical bio-imaging



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ABSTRACT

Carbon dots (CDs) are environmentally benign alternatives to quantum dots comprised of heavy metals with outstanding photoluminescent (PL) properties and have shown great promise in optical bio-imaging and sensing. However, it remains challenging to rationally design and synthesize CDs with red PL emission. We herein disclose that a red-shifted PL emission could be achieved by doping the electron-withdrawing fluorine atoms into CDs. Moreover, we demonstrate the preparation of fluorine doped CDs with a red PL emission under excitation at 530 nm by an easy, environmental friendly, one-step microwave-assistant carbonation route. A possible mechanism of the red-shifted emission upon fluorine doping is tentatively proposed. In addition, the applicable of these red-emissive fluorine doped CDs as optical nanoprobes for bio-imaging applications, both *in vivo* and *in vitro*, was explored. It is indicated that the as-prepared fluorine doped CDs with red-shifted PL emission are promising candidates for tumor bio-imaging/or diagnostics.

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1. Introduction

Carbon-based nanomaterials have received significant attention because of their remarkable properties and potential use in a variety of applications [1–3]. Fluorescent carbon dots (CDs), as a new member in this fascinating family, have shown great promise in bio-imaging and optical sensing by virtue of their outstanding photoluminescent (PL) properties, such as high photo-stability and lack of photo-blinking [4–12]. Another extraordinary aspect of CDs that has attracted considerable attention is the cost-effective and environment-friendly synthesis and excellent biocompatibility, which can hardly be realized with conventional semiconductor quantum dots [13]. These benefits allow CDs to be readily incorporated into biological systems for bio-related applications.

To date, the most common CDs exhibit PL from blue to green while only a few CDs possess PL emission in longer-wavelength regions [14–17] and it remains challenging to rationally design and synthesize CDs with the desired PL properties (e.g. red PL emission), most likely due to a lack of sufficient theoretical and experimental knowledge on the origin of PL in CDs. Nonetheless, there is mounting evidence that the PL emission of CDs originates from both an intrinsic band gap resulting from isolated sp^2 conjugation in the core of CDs and a surface state that can be either directly excited or excited by energy transfer from an intrinsic band [18]. As a consequent, a desirable PL emission of CDs could be achieved either by modifying the surface functional groups and/or by controlling the domain size of sp^2 conjugation.

The surface defects (e.g. oxygen-containing functional groups) serve as capturing centers of excitons and lead to the surface-state-related PL [19]. Increased surface defects by oxidation would narrow the energy levels and consequently cause the red-shifted PL emission of CDs [20–22]. In contrast, blue-shifted PL emission of CDs can be achieved by surface reduction [23]. On the other hand, the band gap of isolated sp^2 domains within the CDs core could function as PL centers as well and the size of sp^2 conjugated fragments determines the band gaps (quantum size effects) [24]. As the size of the fragment increases, the gap gradually decreases and results in a red-shifted PL of CDs [25,26]. Alternatively, incorporation of heteroatoms to the CDs core is another effective strategy to control the PL of CDs. For example, the doping of nitrogen atoms

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into CDs results in a red-shifted PL emission [27–30], which is due to the electron-doping effect of the doped graphitic nitrogen atoms.

It is well documented that introduction of electron-withdrawing fluorine atoms in polyconjugated systems could result in a red-shifted fluorescence [31–33]. Inspired by this, we postulate that introduction of fluorine atoms in addition of nitrogen atoms on the sp^2 conjugated domains in the CDs core might be an effective strategy for further increasing the extent of red-shift. To testify this, we prepared fluorine and nitrogen co-doped CDs through an easy, environmental friendly, one-step microwave synthesis route using citric acid as the carbon source, urea as the nitrogen dopant, and sodium fluoride as the fluorine dopant. These fluorine doped CDs show a red-shifted PL emission compared to that of the CDs without fluorine doping, resulting a red PL under excitation at 530 nm. A possible mechanism is tentatively proposed to explain the behavior of red-shift upon fluorine doping. Moreover, the applicable of these red emissive CDs as optical nanoprobe for bio-imaging was testified, both *in vivo* and *in vitro*.

2. Experimental

2.1. Chemicals

Citric acid, urea, and sodium fluoride were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). 3-(4,5-Dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay kit was purchased from Amresco. Fetal bovine serum (FBS) and Dulbecco's modified Eagle's medium (DMEM) were provided by Gibco (Basel, Switzerland). All other chemicals were of analytical grade and used as received. Double distilled water was used throughout the experiments.

2.2. Synthesis of CDs

A one-step microwave-assisted method was adapted with minor modifications to prepare CDs [34]. Briefly, 180 mg of citric acid, 540 mg of urea and 100 mg of sodium fluoride were added in 10 mL water. The mixture was sonicated and then heated in a domestic microwave oven (750 W, Galanz, China) for 5 min. The obtained CDs were re-dispersed in water and purified by the dialysis against double distilled water using a Spectra/Por dialysis membrane (MWCO = 100–500 Da) for 24 h. Finally, a clear dark yellowish aqueous dispersion containing CDs was obtained for further use.

2.3. Characterization

The morphology and size of CDs were observed by a JEM-2100 F transmission electron microscopy (TEM, JEOL, Japan) at operating voltage of 200 kV. For TEM imaging, one drop of aqueous suspension of the CDs was deposited on a carbon-coated copper grid and dried under vacuum. The size distribution profile of CDs was acquired on a Nano-ZS90 Zetasizer (Malvern, UK). The X-ray diffraction (XRD) pattern of CDs-was obtained on a PANalytical X'pert Pro MPD diffractometer (PANalytical, Holland), using Cu Kα radiation ($\lambda = 1.5405$ Å) at a voltage of 40 kV and a current of 40 mA with 2θ scanning mode. The Fourier-transform infrared (FTIR) spectrum of CDs with a resolution of 4 cm^{-1} was collected over the range of 600–4000 cm^{-1} on an ATR diamond-crystal-equipped miniature FTIR spectrometer (Nicolet iS5 FT-IR spectrometer and iD5 ATR accessory). XPS analysis was performed on an axis ultra-spectrometer (Kratos, UK) using mono-Al Kα line (1486.71 eV) radiation. The UV absorption spectra of CDs were recorded on a UV-3010 spectrophotometer (Hitachi, Japan) using a 1 cm path length cuvette. The room temperature PL emission spectra together with the quantum yield (QY) of CDs were measured on an FLS-980 fluorescence spectrophotometer (Edinburgh Instruments, UK) equipped with an integrating sphere.

2.4. Cytotoxicity assay

C6 glioma cells were seeded in a 96-well plate at 1×10^4 per well and cultured in DMEM medium in a humidified 5% CO_2 incubator at 37 °C for 24 h. Then, the cells were treated with CDs at different concentrations (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg mL^{-1}). After incubation for 24 h, the medium was refreshed and 20 μL of MTT (5 mg mL^{-1}) was added, followed by incubating for 4 h to allow formation of formazan dye. Next, the medium was discarded and DMSO was added into each well. The optical density (OD) value was measured on a Multiskan microplate spectrophotometer (Thermo Fisher Scientific, USA) at a wavelength of 490 nm. The cell viability was assessed by the ratio of OD values between the experimental and control group and was expressed as mean ± standard deviation which deviations from three independent experiments.

2.5. Histopathological examination

Healthy ICR mice were injected with 200 μL of the CDs at a dosage of 20 mg kg^{-1} or with saline as the control. Seven days after injection, the major tissues (heart, liver, spleen, kidneys, lung and brain) were harvested and fixed in 10% formalin solution. The histopathological tests were performed according to standard procedures. In brief, the tissue samples were embedded in paraffin blocks and sectioned into 5 μm slices, followed by hematoxylin and eosin (H&E) staining. The stained sections were observed on an optical microscope (Leica DM4000B) at 10 × magnification.

2.6. In vitro cell imaging

After being seeded on the cover slips at a density of 2×10^4 cells, the glioma C6 cells were cultured in DMEM supplemented with 10% of FBS under humidified 5% CO_2 condition at 37 °C for 12 h. The culture medium was then replaced with FBS-free DMEM containing CDs (0.5 mg mL^{-1}) and the cells were incubated for another 12 h. The cell imaging was performed on a confocal laser scanning microscope (CLSM) with an excitation of 530 nm (Leica TSC SP5).

2.7. In vivo imaging

All animal experiments were performed according to protocols evaluated and approved by the ethical committee of Capital Medical University. Xenograft tumor was established by subcutaneous injection of a suspension of 2×10^6 C6 cells in PBS (60 μL) into the right flanks of male nude mouse (4-week-old, 15–20 g) and was allowed to grow for 8–10 d till the tumor size reached approximately 6 mm. CDs (0.2 mg mL^{-1}, 200 μL) were then intravenously injected into the tumor-bearing nude mouse. The fluorescent imaging were performed at various time points (1, 5, 10, 20, 40, 60, 90 min and 12 h post-injection) on a Kodak *in vivo* FX Pro imaging System (Kodak, USA) using a 530 nm excitation light and a 600 nm emission filter.

3. Results and discussion

3.1. Characterizations

The morphology and size of CDs were examined on the TEM image (Fig. 1a). It was found that the CDs appear as well-separated semi-spherical dots with a size of approximate 10 nm. Fig. 1b shows the high resolution TEM (HRTEM) image of CDs. Note that the CDs exhibits identical well-resolved lattice fringes with a spacing of

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