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Facile and Scalable Preparation of Fluorescent Carbon Dots for Multifunctional Applications

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ABSTRACT

The synthesis of fluorescent nanomaterials has received considerable attention due to the great potential of these materials for a wide range of applications, from chemical sensing through bioimaging to optoelectronics. Herein, we report a facile and scalable approach to prepare fluorescent carbon dots (FCDs) via a one-pot reaction of citric acid with ethylenediamine at 150 °C under ambient air pressure. The resultant FCDs possess an optical bandgap of 3.4 eV and exhibit strong excitation-wavelength-independent blue emission (λ_{Em} = 450 nm) under either one- or two-photon excitation. Owing to their low cytotoxicity and long fluorescence lifetime, these FCDs were successfully used as internalized fluorescent probes in human cancer cell lines (HeLa cells) for two-photon excited imaging of cells by fluorescence lifetime imaging microscopy with a high-contrast resolution. They were also homogenously mixed with commercial inks and used to draw fluorescent patterns on normal papers and on many other substrates (e.g., certain flexible plastic films, textiles, and clothes). Thus, these nanomaterials are promising for use in solid-state fluorescent sensing, security labeling, and wearable optoelectronics.

1. Introduction

With rapid advances occurring in nanomaterials and nanotechnology, many new fluorescent nanomaterials have recently been developed [1], including semiconductor quantum dots [2,3], dye-doped polymeric or silica nanoparticles [4–6], metal nanoclusters [7], rare-earth-doped up-conversion nanoparticles [8,9], and carbon dots (CDs) [10,11]. Among these, CDs, which are also known as carbon quantum dots or carbon nano-dots, are especially attractive due to their low cost, earth abundance, excellent biocompatibility, and resistance to photobleaching [12,13]. Thus far, various potential applications, including biosensing [14], bioimaging [15], photodynamic therapy [16], solar cells [17], light-emitting diodes [18], and catalysts, have been proposed for CDs [19], and have led to a huge amount of literature on the synthesis of fluorescent carbon dots (FCDs), ranging from candle burning to laser ablation and hydrothermal carbonization [20]. One of the frequently used approaches to synthesize bright nitrogen-doped CDs involves hydrothermal reactions of citric acid (CA) with nitrogen-containing bases [21]. For example, CDs with a record high quantum yield (94%) were prepared via hydrothermal treatment of a mixture of CA and ethylenediamine (EDA) at 160 °C [22]. Although hydrothermal methods are very versatile for the preparation of various nanomaterials, they still suffer from multiple disadvantages regarding the commercialization of commodity chemicals, including the high cost of the Teflon-lined stainless steel autoclaves that are used as reactors, the difficulty of

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real-time reaction monitoring due to their closed (or "black-box") reaction processes, and the safety risks that are associated with the high-temperature and high-pressure reaction conditions [23]. Therefore, both for fundamental research and for industrial scale-up, it is important to develop a facile and scalable route for synthesizing high-quality CDs.

Herein, we report a facile and scalable approach to synthesize FCDs by reacting CA with EDA at 150 °C under ambient air pressure. FCDs prepared in this way can be well dispersed in aqueous solution. Having an optical bandgap of 3.4 eV and an excitation-wavelength-independent blue emission (λ_{Em} = 450 nm), the newly developed FCDs were used as internalized fluorescent probes for two-photon excited imaging of cells by fluorescence lifetime imaging microscopy (FLIM) with a high-contrast resolution under 750 nm femtosecond (fs) laser excitation. Compared with the commonly used hydrothermal methods, the methodology developed in the present study has the following advantages: ① It involves much greener reactions that occur at relatively low temperature and ambient pressure [24]; ② it is safer and more scalable for mass-production; and ③ the newly observed excitation-wavelength-independent emission for the resultant FCDs is likely to lead to new research and development opportunities. The FCDs exhibited low cytotoxicity and long fluorescence lifetime, which are promising for bioimaging. Two-photon excited fluorescence imaging in HeLa cells by FLIM was performed using these FCDs as internalized probes. The FCDs were also successfully mixed with commercial inks and retained their fluorescence in the solid state, characteristics that are promising for solid-state fluorescent sensing, security labeling, and wearable optoelectronics.

2. Materials and methods

2.1. Preparation and characterization of fluorescent carbon dots

The FCDs were prepared via reactions of CA with EDA by refluxing the reaction mixture at the ambient air pressure. In a typical procedure, 2 g of CA were dissolved in 3 mL of water within a 50 mL beaker. Next, 3 mL of EDA at room temperature were added. The mixture was heated up (~15 °C·min^{-1}) and maintained at the boiling temperature of water for 30 min. A thermometer was used to monitor the reaction temperature. After the water completely evaporated, the reaction mixture was heated further and transformed from a yellow liquid into a dark brown, soft semisolid, allowing the condensation of CA and EDA to form polymer-like dots. By air-cooling the product down to room temperature, FCDs were obtained as brown solid powder.

A typical high-resolution transmission electron microscopic (HRTEM) image of the FCDs was taken using a Hitachi H-9500 TEM working in bright-field mode. The molecular weights of the FCDs were investigated by polyacrylamide gel electrophoresis (PAGE), using the Pierce™ Prestained Protein Molecular Weight Marker (Thermo Fisher Scientific) as the marker [25]. A PerkinElmer Spectrum GX Fourier-transform infrared (FTIR) spectrometer was used to obtain the FTIR spectra. X-ray photoelectron spectroscopic (XPS; VG Microtech ESCA 2000) measurements were performed using a monochromic aluminum (Al) X-ray source. Thermogravimetric analysis (TGA; TA Instruments) was measured in air with a heating rate of 10 °C·min^{-1}. The absorption spectra of the FCDs were recorded using a Shimadzu UV-1800 ultraviolet-visible (UV-Vis) spectrophotometer. One-photon luminescence and excitation spectra were obtained using a fluorescence spectrophotometer (Hitachi F-2500). Two-photon luminescence spectra excited by a fs laser (750 nm, 150 fs) were recorded using an optical fiber spectrometer (Ideaoptics PG2000). The fluorescent lifetime of the FCDs was measured using a time-correlated single-photon-counting (TCSPC) system with 390 nm excitation and 450 nm emission.

2.2. Cell culture and cytotoxicity studies

HeLa cells were used for *in vitro* studies. The cells were typically cultured in commercial Dulbecco's minimum essential media (DMEM) at 37 °C with 5% CO_2. At 24 h before the *in vitro* cytotoxicity studies, the cells were cultured in 96-well dishes containing about 6000 cells in each well. The FCD samples were then added to each well to achieve a final concentration of 0, 50, 100, 150, 200, 250, and 300 μg·mL^{-1}, respectively. The cytotoxicity of the FCDs after incubation with the cells for 3 h and 24 h was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay according to the manufacturer's protocol. The cells without FCD treatment were considered to be control cells; their viability was estimated to be 100%, and they were used as a reference for the relative viabilities of the cells incubated with FCDs.

2.3. Two-photon excited cell imaging

HeLa cells were used for *in vitro* cell-imaging studies. After the cells were seeded in 35 mm dishes for 24 h, 100 μL of an aqueous solution of FCDs (1 mg·mL^{-1}) was added into the dishes. The cells without FCD additions were considered to be the control groups. After incubation with FCDs for 2h, the cells were washed with 1× phosphate buffered saline (PBS) three times. A confocal laser scanning microscope (CLSM; Olympus FV1000) combined with a fs laser excitation system (mode-locked Ti:sapphire laser system) and a PicoQuant MicroTime 200 FLIM unit were used for the FLIM imaging of the cells.

3. Results and discussion

3.1. Synthesis and characterization of fluorescent carbon dots

Fig. 1(a) illustrates the formation of the FCDs by the reaction of CA and EDA as the starting materials. The temperature of the mixture increased very quickly due to the external heating (~15 °C·min^{-1}) and the heat released from the acid-base neutralization between CA and EDA. After about 5 min heating, the temperature reached the boiling point of water (100 °C), and significant bubble generation was observed due to water evaporation. After the water completely evaporated, the reaction mixture was heated further to 150 °C (measured by a digital infrared thermometer), allowing the condensation of CA and EDA to form polymer-like dots. Spontaneous self-assembling of the polymer-like dots led to nano-sized spherical FCDs. As shown in Fig. 1(b), this newly developed one-pot synthesis approach was also scalable for mass-production. Fig. 1(c) reproduces the HRTEM image for the resultant FCDs, and shows uniform nanoparticles with an average diameter of 3 nm. A corresponding enlarged HRTEM image given in the inset of Fig. 1(c) shows the crystalline structure of the FCDs. The lattice spacing of the FCDs was measured to be 0.205 nm, which is in agreement with the (102) facet of graphite [26]. PAGE studies of the FCDs also indicated that these FCDs have a narrow size distribution and a low molecular weight, as evidenced by the narrow electrophoretic band in the range of 20–25 kDa (Fig. 1(d)).

Fig. 2(a) reproduces the X-ray diffraction (XRD) pattern for the FCDs, which shows a broad peak that is characteristic of CDs with an ultra-small size. The corresponding FTIR spectrum in Fig. 2(b) reveals the characteristic peaks of —COOH (3431 cm^{-1} and 1638 cm^{-1}), —NH (1576 cm^{-1}), and C—NH—C (1125 cm^{-1}). The XPS spectrum of the FCDs given in Fig. 2(c) shows the presence of carbon, nitrogen, and oxygen with atomic percentages of 62.4%, 21.5%, and 16.1%, respectively [27]. As seen in Fig. 2(d), an initial weight loss of 20% was observed by TGA; this loss was caused by the thermal desorption of physically adsorbed water by the FCDs. The subsequent thermo-