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Carbon-dots derived from nanodiamond: Photoluminescence tunable nanoparticles for cell imaging

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ABSTRACT

Water dispersible carbon-dots (CDs) with tunable photoluminescence were synthesized via one-pot hydrothermal oxidation of nanodiamond and subsequently utilized for cell imaging applications. The CDs were characterized by the following techniques including transmission electron microscopy, atomic force microscopy, Fourier transform infrared spectroscopy, UV–Visible spectroscopy, and fluorescent spectroscopy. Results showed that the size of CDs is mainly distributed at 3–7 nm. Many functional groups were introduced on the surface of CDs during hydrothermal oxidation procedure. Cell morphology observation and cell viability measurement demonstrated the good biocompatibility of CDs, suggesting their potential bioimaging applications.

1. Introduction

Over the last decade, fluorescent carbon nanoparticles have attracted great research interest due to their remarkable optical properties, water dispersibility, and biocompatibility [1–3]. Taken advantage of these properties, these nanoparticles have been extensively investigated for bioimaging, biosensing, and catalysts [4–8]. To date, various strategies for the preparation of fluorescent carbon nanoparticles have been developed using organic compounds and carbon materials as carbon precusors [9–15]. In particular, the preparation of fluorescent carbon nanoparticles via one-pot hydrothermal oxidation of carbon materials such as C_{60}, carbon nanotubes, graphite carbon, and graphene oxide has recently been pursed [16–18]. However, to the best of our knowledge, the preparation of fluorescent carbon nanoparticles via hydrothermal oxidation of nanodiamond (ND) has not been reported.

ND is a relative novel class of carbon nanomaterials which can be synthesized on a large scale through detonation method [19,20]. Compared with other carbon nanomaterials, such as carbon black, carbon nanotube, C_{60}, and graphene oxide, ND shows the best biocompatibility to various cells, making it extremely attractive for various biomedical applications [21–35]. Since the first report of the photoluminescence (PL) of ND by Wrachtrup et al., the synthesis and bioimaging applications of fluorescence ND have become one of the most interesting and dynamic research fields [36–40]. Various methods for the preparation of fluorescent ND nanoparticles have thus been developed and extensively investigated for biological imaging in cells and small organisms [41–43]. Among them, the generation of nitrogen-vacancy center in ND by irradiation using a high-energy proton beam and subsequent annealing is the commonly used method for the preparation of fluorescent ND [40]; however, this method suffers from the drawbacks including complex experimental procedure and requirement of expensive equipments. Thus, seeking alternative facile and effective methods for large scale production of fluorescent ND is desirable. Recently, Mochalin and Gogotsi have reported the wet chemistry route for preparation of fluorescent ND by conjugating carboxylated ND nanoparticles with hydrophobic octadecylamine [44]. Compared with the traditional irradiation approach, this wet chemistry method is relative simple and effective, opening up a new door for preparation of fluorescent ND. However, the octadecylamine conjugated ND nanoparticles cannot be dispersed in aqueous media, which severely limited their biomedical applications.

In current work, we reported for the first time, the preparation of water dispersible CDs via one-pot hydrothermal oxidation of ND. The cell imaging applications and biocompatibility of the CDs were also investigated (Scheme 1). Compared previous reports, the method described in this work for preparation of ND based fluorescent CDs is rather simple and effectiveness. Thus, obtained CDs showed tunable PL, excellent water dispersibility, and biocompatibility, which are promising for cell imaging applications.

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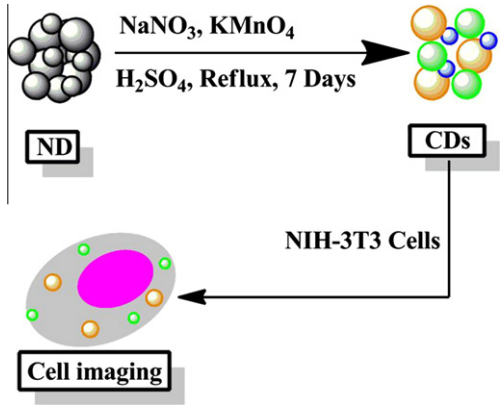

Scheme 1. Diagram showing the preparation of CDs via one-pot thermal oxidation ND and the use of CDs for cell imaging. CDs show excitation-dependent PL due to the wide size distribution of CDs.

2. Experimental procedure

2.1. Materials and characterization

ND was purchased from Beijing Grish Hitech Co. Ltd. (Beijing, China). Its diameter is about 30 nm according to the supplier. All the other chemical agents were obtained from commercial routes with at least analytical grade. CDs were prepared by hydrothermal oxidation of ND powder using modified hummers method [45]. Briefly, ND (1.0 g), $NaNO_3$ (6.0 g), and $KMnO_4$ (3.0 g) were added to concentrated sulfuric acid (48 mL) successively under stirring in ice bath. Then, 100 mL of deionized water was poured into the mixture. And then, the mixture was refluxed for 7 days. For obtained the fluorescent particles, the dialysis tube against with ethanol, the molecular weight cutoff between 1000 and 7000 was collected and for further characterization.

The fluorescence measurement was made on a PE LS-55 spectrometer equipped with quartz cuvettes of 1 cm path length. The Fourier transform infrared (FT-IR) spectra were obtained in a transmission mode on a Perkin–Elmer Spectrum 100 spectrometer (Waltham, MA, USA). Typically, 4 scans at a resolution of 1 cm^{-1} were accumulated to obtain one spectrum. UV–Visible absorption spectra were recorded on a UV/Vis/NIR Perkin–Elmer lambda750 spectrometer (Waltham, MA, USA) using quartz curettes of 1 cm path length. Transmission electron microscopy (TEM) images were recorded on a TECNAIG2 20 microscope operated at 200 kV. The TEM specimens were made by placing a drop of the nanoparticle suspension on a carbon-coated copper grid. Atomic force microscopy (AFM) images were taken out using a Nanoscope multiMode SPM (Digital Instruments) with a AS-12 scanner operated in tapping mode IN conjunction with a V-shaped tapping tip (Applied Nanostructures SPM model: ACTA). The images were recorded at a scan rate of 2 Hz. The X-ray photoelectron spectra (XPS) were measured using a PHI Model Quantera SXM scanning X-ray microprobe, using Al Kα as the excitation source (1486.6 eV), and binding energy calibration was based on C1s at 284.8 eV. The zeta-potential of CDs in phosphate buffer saline (PBS), fetal bovine serum (FBS) free, and FBS containing cell culture medium were determined using a zeta Plus particle size analyzer (ZetaPlus, Brookhaven Instruments, Holtsville, NY).

2.2. Cytotoxicity of CDs

2.2.1. Observation of cell morphology incubated with CDs

Mouse embryo fibroblast (NIH-3T3) cells and adenocarcinomic human alveolar basal epithelial (A549) cells were cultured in Dubecco's modified eagle medium (DMEM) supplemented with 10% heat-inactivated FBS, 2 mM glutamine, 100 U mL^{-1} penicillin, and 100 μg mL^{-1} of streptomycin. Cell culture was maintained at 37 °C in a humidified condition of 95% air and 5% CO_2 in culture medium. Culture medium was changed every 3 days for maintaining the exponential growth of the cells.

The effect of CDs on NIH-3T3 and A549 cells was first examined by optical microscopy. Briefly, cells were seeded in 6-well microplates at a density of 1×10^5 cells mL^{-1} in 2 mL of respective media containing 10% FBS. After cell attachment, plates were washed with PBS, and the cells were treated with complete cell culture medium, or cell culture media containing 50 μg mL^{-1} of CDs for 6, 12, 24, 36, 48 h. Then, all samples were washed three times with PBS to remove the uninternalized nanoparticles. Cells were observed by using an optical microscopy (Leica, DMI3000B, Germany), and photos were taken using a charge-coupled device (CCD). The overall magnification was ×100.

2.2.2. Cell viability assay

Cell viability of CDs on NIH-3T3 and A549 cells was determined by counting kit-8 (CCK-8) assay according to our previous report [29,46]. Briefly, cells were seeded in 96-well microplates at a density of 5×10^4 cells mL^{-1} in 160 μL of respective media containing 10% FBS. After 24 h of cell attachment, the cells were incubated with 20, 40, 80, 160, 320 μg mL^{-1} of CDs nanoparticles for 24 h. Then nanoparticles were removed, and cells were washed with PBS for three times. 10 μL of CCK-8 dye and 100 μL of DMEM cell culture media were added to each well and incubated for 2 h at 37 °C. Plates were then analyzed with a microplate reader (VictorIII, Perkin–Elmer). Measurements of dye absorbance were car-

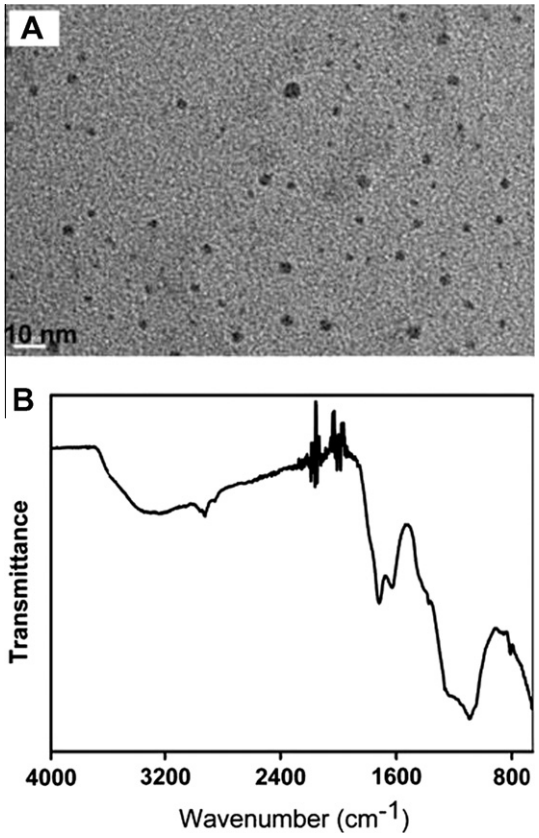

Fig. 1. (A) Representative TEM image of CDs. Scale bar = 10 nm. (B) FT-IR spectrum of CDs, stretch bands centered at 3400, 1732, and 1100 cm^{-1} were observed, suggesting the functional groups including —COOH, —OH and C—O were existed on the surface of CDs.