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Improving the biocompatibility of carbon nanodots for cell imaging

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ABSTRACT

In the practice of in vivo imaging with carbon nanodots (CNDs) as probe, the volume of CNDs solution introduced into living body should be kept at minimum, and a higher concentration is needed to ensure sufficient quantity of the probe for obtaining bright image. Therefore, the improvement on biocompatibility of the CNDs is among the most important and critical issues. We report herein the improvement on the biocompatibility of CNDs with modification by ionic liquid. Amide group functionalization of carbon nanodots is first conducted through microwave irradiation, followed by coupling the ionic liquid 1-carboxymethyl-3-methyl imidazolium bromide on the surface of the Amide-CNDs via covalent conjunction to produce the modified carbon nanodots (IL-CNDs). This modification process significantly improved the biocompatibility of CNDs, as demonstrated by cell imaging at a higher concentration of CNDs. Both Amide-CNDs and IL-CNDs exhibit abundant surface functional groups, resulting in tunable fluorescent emission feature and potential applications in two-color cell imaging.

1. Introduction

Non-toxic and non-bleaching fluorescent carbon nanodots (CNDs) have attracted widespread attention as an alternative to semiconductor-based quantum dots (QDs) and organic dyes [1]. CNDs with sizes below 10 nm have become an important member of carbon nanomaterials since its first discovery in 2004 [2,3]. Due to the high solubility, facile modification feature, and special fluorescent property, CNDs show high potentials in biomedical, optronic and catalytic applications as well as biosensing and bioimaging investigations [4–18]. A notable feature of CNDs is the excitation-dependent fluorescent emission, and the tunable fluorescent property related to surface functionalization groups [19,20].

CNDs are usually prepared via either top-down or bottom-up approaches [21]. Element doping and surface modifications are usually employed to improve the fluorescent property. CNDs doped with metals, e.g., zinc, and nonmetals, e.g., boron, nitrogen, sulfur and phosphorus, exhibit enhancement on the quantum yield or shift on the maximum excitation/emission wavelength [22–26]. The wavelength of emission light varied with surface groups which introduce different trapping states with various energy levels. As illustrated by the red-shift fluorescent emission of CNDs with higher surface oxidation and the blue-shift of fluorescent emission after reduction [20,27]. For this purpose, polyethylenimine (PEI) and polyethylene glycol (PEG) or their derivatives are frequently used as surface modification agents for nanomaterials. It has been reported that CNDs passivated with poly(propionylethylenimine -co-ethylenimine) (PPEI-EI) and PEG exhibit obvious improvement on the solubility as well as fluorescence [28], and a red shift on the fluorescent emission of CNDs is observed after modification with PEI [29]. A simple one-pot procedure is reported with PEI as both carbon source and passivation agent for the production of pH sensitive CNDs [30]. There are also reports on the application of PEI functionalized CNDs for bioimaging and nucleic acid delivery, although the cell cytotoxicity of PEI-CNDs is higher than that of CNDs functionalized with other surface groups, e.g., PEG or carboxylic groups [31–33].

Ionic liquids (IL) are generally organic salts consist of cationic and anionic moieties which provide flexible alternatives for the regulation of their physicochemical properties. The unique features of ionic liquids, e.g., negligible vapor pressure and high chemical/thermal stability, endow them favorable biocompatibility and make them highly suitable for biological related studies, e.g., extraction/isolation of macrobiomolecules [34,35] and biocatalysis [36]. Recently, ionic liquid has been used for the functionalization of CNDs via electrochemical exfoliation of graphite, which find applications in electrocatalysis, biosensing and bioimaging [37,38]. Ionic liquid modified CNDs obtained via hydrothermal treatment show tunable amphiphilicity and multi-functional applications, e.g., ion sensing and fluorescent ink [39].

In the present study, an ambient-temperature approach is reported for the functionalization of carbon nanodots (IL-CNDs) by

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using ionic liquid 1-carboxymethyl-3-methylimidazolium chloride (CmimCl), through covalent coupling between the carboxyl ionic liquid and the amide groups functionalized carbon nanodots (Amide-CNDs). The obtained IL-CNDs exhibit obvious change on the fluorescent emission feature and their biocompatibility has been significantly enhanced, which facilitate bioimaging applications.

2. Materials and methods

2.1. Materials and reagents

D(+)-Glucosamide hydrochloride, branched polyethylenimine (B-PEI) are purchased from Sigma-Aldrich (Milwaukee, USA). N-hydroxysuccinimide (NHS), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC · HCl) and 2,4,6-trinitrobenzenesulfonic acid are obtained from Aladdin Reagent Co. Ltd. (Shanghai, China). Ionic liquid used in the present study, e.g., 1-carboxymethyl-3-methylimidazolium chloride (CmimCl), is the product of Cheng Jie Chemical Co. Ltd. (Shanghai, China). Diethylenetriamine (DTM), dimethyl sulfoxide (DMSO), HCl, NaOH, NaCl, KCl, Na_2HPO_4 and KH_2PO_4 are obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Fetal bovine serum, Dulbecco's modified Eagle's medium (DMEM), trypsin (0.25%), penicillin, streptomycin are purchased from Hyclone (Logan, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay kit is obtained from Nanjing KeyGEN Biotech Co. Ltd. (Nanjing, China). All the reagents used are at least of analytical reagent grade unless otherwise specified. Deionized water (DI water) of 18 MΩ cm is used throughout.

2.2. Preparation of the ionic liquid modified carbon nanodots

Amide functionalized carbon nanodots (Amide-CNDs) are prepared firstly via a microwave procedure with glucosamine as a carbon source, B-PEI and DTM serve as passivation agents. Typically, 5 mL glucosamine solution (0.2 g mL^{-1}), 2 mL B-PEI solution (0.25 g mL^{-1}), 400 μL DTM and 8 mL pure water are mixed and sealed into a 100-mL Teflon digestion tank and heated at 200 °C for 15 min in a COOLPEX Microwave Chemical Reaction Apparatus (PreeKem Scientific Instruments Co. Ltd., China). After cooling down to ambient temperature, the solution is then dialyzed over deionized water in a dialysis bag (MWCO membranes 3500 Da) for 48 h. The solution in the dialysis bag is then lyophilized. The obtained product is dispersed in pure water at a concentration of 15.3 mg mL^{-1}.

Ionic liquids modified carbon nanodots (IL-CNDs) are prepared via covalent bonding interaction. Typically, 153 mg CmimCl, 830 mg EDC · HCl, and 1.24 g NHS are added into 10 mL PBS buffer solution (10 mM, pH 5.5). The mixture is magnetically stirred for 15 min. Subsequently, 1 mL Amide-CNDs solution (15.3 mg mL^{-1}) is added. Afterwards, pH value of the mixture is adjusted to 7.4 with sodium hydroxide, and the reaction is allowed to continue for 12 h under stirring. The solution is then dialyzed against water in a dialysis bag (MWCO membranes 1000 Da) for 72 h followed by lyophilization. It is dispersed in water and the concentration of the obtained product is derived to be 100 mg mL^{-1}. This process is illustrated in Scheme 1.

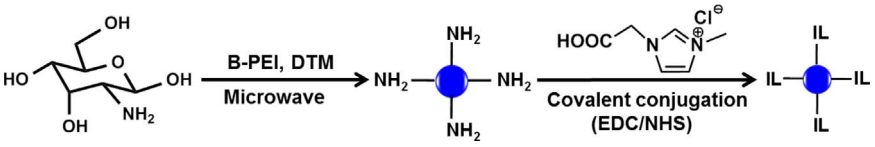
A PB-10 pH meter (Thermo Instruments Inc., USA) is used to measure the pH values. UV–vis spectra are recorded on a U-3900 UV–vis spectrophotometer (Hitachi High Technologies, Japan). Photoluminescence behavior is evaluated by an F-7000 fluorescence spectrophotometer (Hitachi High Technologies, Japan) and quantum yields are quantified with a Fluormax 4 fluorescence spectrophotometer (Horiba Scientific, France). Surface charge analysis for the carbon nanodots is conducted by using a nano ZS-90 particle size analyzer (Malvern Instruments, United Kingdom). A Nicolet-6700 spectrophotometer (Thermo Instruments Inc., USA) is employed to record the FT-IR spectra. X-ray diffraction (XRD) patterns are recorded on a MPDDY2094 X-ray diffractometer (PAnalytical B.V, Netherlands) with Cu-Ka irradiation (λ=1.5406 Å) in the range of 2θ from 10 to 80°. Transmission electron microscopy (TEM) images are obtained using a JEM-2100 high resolution transmission electron microscope (JEOL, Japan). HeLa cells are cultured in a HERA Cell 150 incubator (Thermo Instruments Inc., USA), and their images are obtained on an inverted fluorescent microscope (Nikon, Japan). MTT assay is conducted by using a Synergy H1 ELISA plate reader at 490 nm (BioTek, USA).

2.3. Cytotoxicity and cell imaging

HeLa cells are cultured in DMEM medium supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) penicillin/streptomycin at 37 °C in a humidified environment of 5% CO_2. In a typical culture procedure, HeLa cells are first inoculated in T-flasks with a culture base area of 25 cm^2 and cultured for 48 h. They are digested with 1 mL trypsin (0.25%) solution for 1 min, and then centrifuged at 800 rpm for 2 min. The supernatant is replaced by 1 mL PBS solution, followed by HeLa cell counting with a blood counting chamber. Usually, cell density is in the range of 2–3 × 10^6/mL at the present culture conditions. Afterwards, the PBS suspension of HeLa cells are centrifuged at 800 rpm for 2 min, and the supernatant is replaced by 1 mL DMEM medium supplemented with FBS and penicillin/streptomycin.

MTT is employed to test the cytotoxicity of carbon nanodots as illustrated in our previous report [40]. In a typical MTT assay, the DMEM suspension of HeLa cells is diluted using DMEM medium supplemented with FBS and penicillin/streptomycin, and 100 μL of which (containing ca. 7000–10000 cells) is added to each well of a 96-well plate. The cells are cultured for 12 h, and the culture medium is then replaced by fresh portion containing CNDs of various concentrations. After further culture for 20 h, 20 μL of MTT solution (5 mg mL^{-1}) is added into each well, followed by culture for 4 h. The culture medium is then replaced by 150 μL DMSO. After mechanical shaking for 5 min in the Synergy H1 ELISA plate reader, the absorbance (A) at 490 nm of the mixture is recorded, and the cell viability is derived.

For imaging studies, the HeLa cells are inoculated in glass T-flasks and cultured for 24 h, and the culture medium is then replaced with new portion containing various concentrations of Amide-CNDs and IL-CNDs. After incubation for 12 h, the culture medium is removed and the cells are washed thoroughly with phosphate buffer (PBS, 10 mmol L^{-1}, pH 7.4). The HeLa cell imaging is finally recorded by using an inverted fluorescence microscope with excitation/emission wavelength at 330–380/420 nm



Scheme 1. Schematic illustration for the preparation of IL-CNDs.