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Ionic liquid mediated organophilic carbon dots for drug delivery and bioimaging

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ABSTRACT

By taking advantage of the structural tunability of ionic liquids (ILs), a series of imidazolium ILs were employed as precursors to prepare carbon dots (IL-CDs) and as regulators to control their properties. The simultaneous formation of hydrophilic CDs (IL-HCDs) and organophilic CDs (IL-OCDs) is achieved in hydrothermal reaction system by undergoing sulfuric acid carbonization. The quantum yields (QY) of IL-OCDs are closely correlated with both the cationic and anionic moieties of the ionic liquids, i.e., longer side chains of cations in the imidazolium ILs and weakly nucleophilic anions tend to produce highly fluorescent IL-OCDs. Both IL-HCDs and IL-OCDs exhibit low cytotoxicity, and that of IL-HCDs is even lower than IL-OCDs. A drug delivery system is constructed by combining anticancer drug curcumin (Cur) with IL-OCDs via hydrophobic interaction, among which 1,3-dibutylimidazolium nitrate derived IL-OCDs exhibit highest photoluminescence. In addition, it serves as a favorable drug carrier with high drug loading efficiency and facilitates rapid penetration/transportation of Cur into the cell interior, which significantly accelerates the apoptosis of HeLa cells. This process is further visualized by cell imaging.

1. Introduction

Fluorescent carbon nanoparticles (carbon dots, CDs) have shown promising potentials in bio-imaging and drug delivery due to their low toxicity, favorable photostability and ease of surface functionalization [1–3]. Most CDs reported so far are hydrophilic in nature, while organophilic/hydrophobic CDs (OCDs) with good solubility are highly desired for studies in hydrophobic environment, e.g., cell membrane. In terms of drug delivery, many anticancer drugs are hydrophobic, OCDs is an exclusive and suitable alternative as nanomedicine carrier to improve the drug loading rate [4–6], and facilitate fast penetration of cell membrane which ensure high drug delivery efficiency However, studies on OCDs is far less than that for hydrophilic CDs (HCDs). A few approaches were reported for the preparation of OCDs, where hydrophobic long-chain organic molecules, e.g., dodecanethiol, hexadecylamine and poloxamer, are predominantly used as surface passivation/capping agents [7–9]. Another strategy is the modification of HCDs surface with hydrophobic moietyto tune/regulate the hydrophobicity of the CDs [10].

As is known that ionic liquids (ILs) consist of organic cations and organic/inorganic anions [11–13]. The intrinsic non-molecular natures of ILs give rise to unique physico-chemical properties. Especially their cations and anions are diverse and changeable, which makes it feasible to precisely adjust/regulate the characters of ILs. By taking advantage of this merit, ILs have been used as carbon sources for the preparation of ionic liquid mediated CDs, by microwave-assisted reaction, hydrothermal treatment as well as sulfuric acid carbonization [14–18]. The hydrophilicity/hydrophobicity of the obtained CDs can be readily tuned/regulated by anion exchange of the ionic liquid moiety grafting on CDs surface [10]. In our previous work, a one-pot procedure was developed for the preparation of both IL-HCDs and IL-OCDs with 1-butyl-3-methylimidazolium hexafluorophosphate as the carbon source in a H_3PO_4-ethanol medium [19]. The mass proportion of HCDs to OCDs is simply regulated by varying the H_3PO_4/ethanol molar ratio. It is further found that the modification of CDs with ILs significantly improved their biocompatibility [20], and IL-OCDs penetrate the cell membrane via multiple pathways, which facilitate fast

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penetration of IL-OCDs into HeLa cell interior within 1 min [21].

In biological studies, especially bioimagings, the fluorescent quantum yield of CDs is highly important to achieve bright and clear images for biological cells. The above studies indicated that ionic liquids have been applied for regulating the hydrophilicity/hydrophobicity of CDs, while so far there is no report on the demonstration of regulating the quantum yield of CDs with ionic liquids. In the present study, 11 imidazolium ILs were used as precursors for the preparation of IL-CDs, meanwhile, ILs serve as regulators to control the properties of the obtained CDs. The length of side chain of the imidazolium cation and the structure of anions in the ionic liquids pose significant influence on the fluorescence behavior, and cell imaging capacity of the obtained CDs. Furthermore, organophilic fluorescent CDs have been demonstrated to be a suitable drug delivery carrier and exhibit favorable biocompability with high drug loading efficiency, which ensures rapid penetration of the CDs into the cell interior and facilitates cell imaging therein.

2. Experimental

2.1. Chemicals and reagents

The following ionic liquids are purchased from Shanghai Cheng Jie Chemicals Co. LTD (Shanghai, China), i.e., 1-ethyl-3-methylimidazolium tetrafluoroborate ($EMIMBF_4$), 1-butyl-3-methylimidazolium tetrafluoroborate ($BMIMBF_4$), 1-hexyl-3-methylimidazolium tetrafluoroborate ($HMIMBF_4$), 1-octyl-3-methylimidazolium tetrafluoroborate ($OMIMBF_4$), 1-decyl-3-methylimidazolium tetrafluoroborate ($DMIMBF_4$), 1,3-dibutylimidazolium chlorine (BBIMCl), 1,3-dibutylimidazolium bromine (BBIMBr), 1,3-dibutylimidazolium nitrate ($BBIMNO_3$), 1,3-dibutylimidazolium tetrafluoroborate ($BBIMBF_4$), 1,3-dibutylimidazolium hexafluorophosphate ($BBIMPF_6$) 1,3-dibutylimidazolium bis((trifluoromethyl)sulfonyl)imide ($BBIMTf_2N$). Curcumin (Cur), sulfuric acid is obtained from Sinopharm Chemical Reagent (Shenyang, China) and used as received. Dulbecco's modified medium (DMEM, high glucose), trypsin, penicillin/streptomycin and fetal bovine serum are obtained from Thermo Scientific (Logan, Utah, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay kit is the product of Nanjing KeyGEN Biotech (Nanjing, China). Other chemicals employed are at least of analytical reagent grade. Deionized water of 18 MΩ cm^{-1} is used throughout the experiments.

2.2. Instrumentations

High-resolution transmission electron microscopy (HR-TEM) images of the CDs were recorded on a JEM-2100 F microscope (JEOL, Japan) operating at 200 kV. The specimen was prepared by drop-casting the sample aqueous solution (0.1 mg/mL) onto a carbon-coated copper grid, followed by drying at room temperature. Fourier transform infrared (FT-IR) spectra were obtained on a Nicolet-6700 spectrophotometer (Thermo Instruments Inc., USA) by using KBr pellets. UV–vis absorption spectra were recorded using a JASCO V-550 UV/visible spectrophotometer (JASCO International Co., Ltd., Tokyo, Japan). Fluorescence spectra were performed on a F-7000 fluorescence spectrophotometer (Hitachi High Technologies, Japan) providing a 0.5 cm quartz cuvette. The quantum yields of the CDs were recorded on a Quantaurus-QY absolute photoluminescence quantum yield measurement system (Hamamatsu Photonics, Japan). X-ray diffraction (XRD) patterns were recorded on a MPDDY2094 X-ray diffractometer (PAnalytical B.V., Netherlands) with Cu-Kα irradiation (λ 1.5406 Å). X-ray photoelectron spectroscopy (XPS) spectra were conducted with an ESCALAB 250 surface analysis system (Thermo Instruments Inc, USA). Cell imaging studies were performed on a fluorescence micro-imaging system (UltraVIEW VoX, Perkin Elmer, America).

2.3. Preparation of ionic liquid mediated IL-CDs

Ionic liquid mediated carbon dots (IL-CDs) were prepared by sulfuric acid carbonization of the corresponding ionic liquids. 1 g of a specific ionic liquid was added into 4 mL of H_2SO_4-ethanol solution (12%, m/m) in a 25-mL hydrothermal reactor. The mixture is transferred into an electric thermostatic drier and kept at 200 °C for 48 h. After cooling down to room temperature, the resultant dark brown solution was neutralized with fresh NaOH-ethanol solution. The sediment was removed by 10000 rpm centrifugation for 10 min. The supernatant was dialyzed by molecular weight cut-off (MWCO) of 100–500 against ultrapure water for 72 h. The hydrophilic CDs (HCDs) dissolved in water and were obtained by freeze drying of the aqueous medium, while the organophilic CDs (OCDs) gathered in the inner surface of the dialysis bag and were collected by decanting the HCDs aqueous solution. Finally the OCDs were dialyzed against ethanol to remove the impurity and dried under vacuum. HCDs and OCDs were dissolved in ethanol for further use.

2.4. Cell incubation and MTT assay

HeLa cells were cultured in Dulbecco's modified medium (DMEM) supplemented with 10% fetal bovine serum and 1% antibiotics penicillin and maintained at 37 °C with 5% CO_2.

HeLa cells within replicate 96-well plates were pre-cultured at standard culture conditions for 24 h. After removal of the medium, cells were incubated with fresh media containing IL-CDs (100 μg mL^{-1} for IL-OCDs, 200 μg mL^{-1} for IL-HCDs) for extra 20 h. 20 μL of MTT solution (5 mg mL^{-1}) was then added to each well, and after incubated for 4 h, the MTT solution was removed. 100 μL of DMSO was added into each well to dissolve the formed crystals. The absorbance was measured at 490 nm using a Polarstar microplate reader.

2.5. Loading of curcumin on $BBIMNO_3$ oriented OCDs

For the loading of anticancer drug Cur, 80 μg mL^{-1} Cur ethanol solution was mixed with 20 μg mL^{-1} $BBIMNO_3$ oriented organophilic OCDs in ethanol. The resulting Cur/OCDs nanocomposite was collected by dialysis against 50% ethanol aqueous solution to remove the free or unadsorbed Cur (Cur in the solution not retained by the OCDs). The outside dialysis solution was collected and the concentration of Cur is determined by measuring the absorbance at 425 nm to evaluate the loading ratio of Cur, and the mass of unadsorbed Cur (mu_{Cur}) is derived. The loading ratio of Cur onto OCDs is expressed as: $(m_{Cur}-mu_{Cur})/m_{CDs} \times 100\%$, with m_{Cur} and m_{CDs} as the mass of original Cur and OCDs, respectively.

3. Results and discussion

3.1. Preparation and characterizations of the ionic liquid mediated CDs

3.1.1. The yields of IL-HCDs and IL-OCDs

11 imidazolium ILs were chosen as precursors to prepare the IL-CDs for the purpose of evaluating their effect on the properties of IL-CDs. The ILs used in this study were divided into 2 categories. The first group included 5 ILs with the same anion [BF_4^-] and different side chain of cations ($EMIMBF_4$, $BMIMBF_4$, $HMIMBF_4$, $OMIMBF_4$, $DMIMBF_4$). The second group contained 6 ILs with the same cation [$BBIM^+$] and various anions (BBIMCl, BBIMBr, $BBIMBF_4$, $BBIMPF_6$, $BBIMTf_2N$). The structures and water solubilities of these