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Development of multicolor carbon nanoparticles for cell imaging

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ABSTRACT

Multicolor carbon nanoparticles (CNPs) were prepared, characterized and developed as fluorescent probes for cell imaging. The fluorescent CNPs were prepared with a facile hydrothermal oxidation route by using linear polysaccharide cellulose and cyclic oligosaccharide cyclodextrin as carbon sources. The characterizations by transmission electron microscopy show that the prepared cellulose-CNPs and cyclodextrin-CNPs are spherical, well-dispersed in water with average diameters of 100 nm and 76 nm, respectively. Under the excitation of UV light, the CNPs are strongly luminescent with an excitation-dependent emission behavior and bathochromic emission properties. The fluorometric methods show that the cellulose-CNPs and cyclodextrin-CNPs are strongly fluorescent with fluorescence quantum yield of 7.47% and 4.49%, respectively. The multicolor CNPs have excellent photostability toward photobleaching. Strong near-infrared fluorescence of the carbon nanoparticles was observed with a 632.8 nm excitation wavelength laser. The oxidative metal ions like Hg(II), Cu(II) and Fe(III) show an quench effect on the fluorescence intensity of the CNPs. The multicolor CNPs were successfully used as fluorescent probes for mouse melanoma cells imaging. The results indicate that the multicolor CNPs derived from cellulose and cyclodextrin may have a great potential for the applications in bioimaging.

1. Introduction

Fluorescent semiconductor quantum dots (QDs) have been extensively used in biological applications, such as bio-molecular labeling, contrast agents, bio-sensing and drug delivery.[1–3] However, potential toxicity from heavy metals has drawn great attention on serious safety concern and environmental risks.[4,5] For this reason, various fluorescent nanoparticles made from alternatives materials with similar optical properties have been investigated as candidates for potential applications in optical imaging.[6–8] For example, we developed a method to prepare the lanthanide chelate-doped silica nanoparticles for highly sensitive time-resolved immunoassay[9–11]. The use of silica-doped fluorescent nanoparticles can improve the photostability and detection sensitivity of the nanoparticles. However, tedious synthesis steps and surface activation procedure of the nanoparticles were required for their use in bio-molecule labeling and cell imaging[9].

Carbon-based nanomaterials like carbon nanotubes, fullerenes, nanofibers and graphene nanosheets have been drawn great attention for their applications in nanosensors, electrical devices, catalysis reactors, drug delivery and electrochemistry.[12,13] Recent studies have shown that a new class of carbon nanoparticles (CNPs) exhibit promising fluorescent properties, such as high photostability, tuneable excitation and emission wavelength, low toxicity, good biocompatibility and bright electroluminescence.[14–18] The major element of CNPs, carbon, is hardly considered as a toxic substance compared with the heavy metals from conventional QDs. Because of these features, various methods for fluorescent CNP synthesis have been reported by using carbon-based materials as carbon resources.[14,19,20] For example, top-down methods include laser ablation of carbon powder[14], electrochemical oxidation[21] and arc discharge[22]; bottom-up methods consist of microwave synthesis,[23,24] combustion soots of candles,[25,26] thermal oxidation of carbon precursor by employing silica or zeolites as carriers[27,28] commercial activated carbon[29], lampblack[30] and watermelon peel as carbon resources[31]. All these methods suffer to some degree from drawbacks like tedious processes, harsh synthetic conditions or expensive starting materials. Preparation of fluorescent CNPs from bioprecursors may provide a new approach for bottom-up CNPs fabrications. Although carbon nanoparticles derived from

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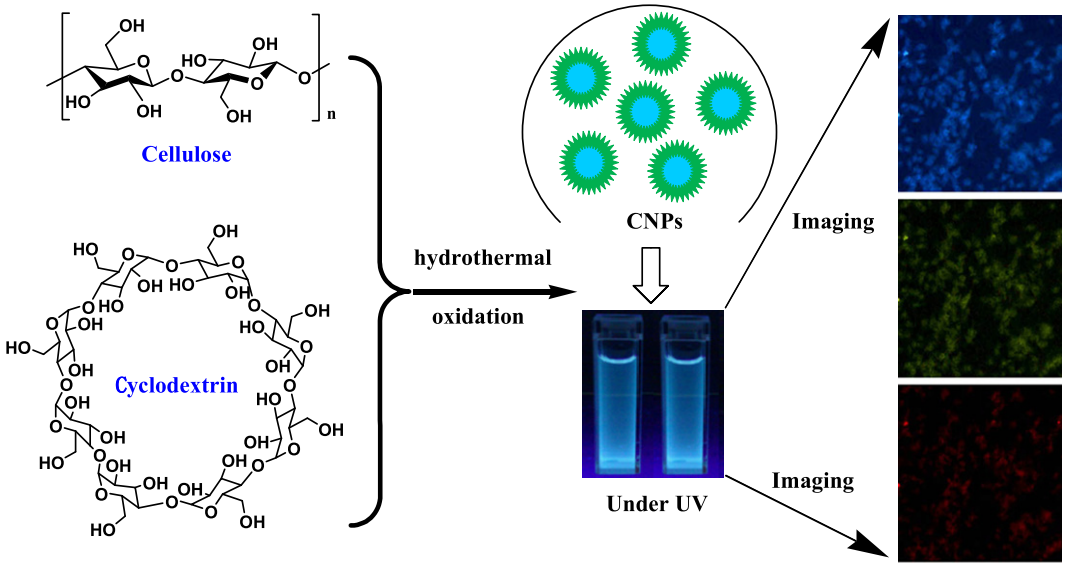

Scheme 1. Preparation scheme of multicolor CNPs from cellulose and cyclodextrin. (For interpretation of the references to color in this scheme legend, the reader is referred to the web version of this article.)

glucose and sucrose have been reported, [20,32] to the best of our knowledge, the direct synthesis of fluorescent CNPs from linear polysaccharides and cyclic oligosaccharides and their fluorescent properties remains less studied.

Herein, we report a one-step method of alkali-assisted hydrothermal oxidation of polysaccharide cellulose and cyclic oligosaccharide cyclodextrin, to prepare multicolor fluorescent CNPs (Scheme 1). The CNPs that were obtained emit bright and colorful fluorescence covering the whole visible spectral range. The multicolor CNPs are highly water-soluble, nano-sized (100 nm, 76 nm) and quite stable against photo-bleaching as compared with conventional organic dyes. Strong near infrared fluorescence of the CNPs was detected using a 632.8 nm laser as excitation source. The oxidative metal ions like Hg(II), Cu(II) and Fe(III) displayed an quench effect on the fluorescence intensity of the CNPs. The multicolor CNPs were successfully used as fluorescent probes for mouse melanoma cells imaging. The developed multicolor CNPs derived from saccharides may have a potential for the applications in biomedical fields.

2. Experimental

2.1. Materials and instrumentation

Cellulose was purchased from Fluka (Avicel PH-101) and cyclodextrin was purchased from Chemical Reagent Co. Ltd. (Tianjin, China). Dialysis bag was purchased from Genestar Biotechnology Co. Ltd. (Shanghai, China). Mouse melanoma cell line B16-F10 was purchased from KeyGen Biotechnology Co. Ltd. (Nanjing, China).

A JEOL model JEM-2000EX transmission electron microscope was used for measuring the shape and size of the nanoparticles. UV–vis absorption spectra were measured on a Shimadzu UV2550 UV–vis spectrophotometer. Fourier transform infrared spectra were performed using a VECTOR 22 FTIR spectrometer with a KBr pellets. Fluorescence spectra and emission lifetime were measured on a Perkin–Elmer LS 55 spectrofluorometer. Zeta potential was determined by laser Doppler velocimetry at 25 °C using a Nano ZS90 Zetasizer (Malvern Instruments, Malvern, U.K.). NIR emission spectra were measured by Princeton Instrument Acton SP2500 Spectrograph with a PIXIS 100 CCD equipped with a 632.8 nm He-Ne laser (model, 25 LHP 928-230; power, 25 mW) as an excitation source. The integration time for cellulose-CNPs and cyclodextrin-CNPs are 1 ms and 500 ms, respectively. Fluorescence imaging measurements of cell labeling were performed on a inverted Nikon Te-2000 U microscope with an excitation filter of 330–380 nm, 450–490 nm and 510–560 nm for blue, green and red color respectively. *Ex vivo* imaging was carried out with a CRi Meastro Ex *in vivo* imaging system (Caliper Life Sciences Inc. U.S.A.).

2.2. Preparation of carbon nanoparticles (CNPs)

Multicolor CNPs were synthesized directly from alkali-assisted hydrothermal oxidation of cellulose or cyclodextrin aqueous solution according to a modified procedure as previously described. [32] Briefly, 2.0 g of cellulose or cyclodextrin and 0.2 g of sodium hydroxide were added to 30 ml of water with vigorous stirring. The mixture solution was then sealed into a Teflon-lined stainless-steel autoclave and heated at a constant temperature of 160 °C for 4 hours. Then, the solution was cooled at room temperature and the dark brown solution was obtained. The multicolor CNPs were isolated by centrifuge at a speed of 10000 rpm for 20 min to remove the deposit. The resulting CNPs were neutralized by 1 mol/L hydrochloric acid solution and extensively dialyzed against distilled water through a dialysis membrane with a molecular weight cutoff of 3000 to remove the impurities. The multicolor CNPs were purified with Sephadex G25 column and dried in a lyophilizer for the following use.

2.3. Metal ions effects on fluorescence intensity

Standard aqueous solutions (10^{-4} mol/L or 10^{-6} mol/L) of $CaCl_2$, $MgCl_2 \cdot 6H_2O$, $CuSO_4 \cdot 5H_2O$, NaCl, K_2CO_3, $ZnCl_2$, $NiSO_4$ $6H_2O$, $FeCl_3$, Hg_2Cl_2 and $CdCl_2$ were prepared in distilled water. To 4.5 ml of standard metal ions solution was added 0.5 ml of the multicolor CNPs solution (0.5 mg/ml) and the mixture was stirred at room temperature. The fluorescent intensity of mixed solution was measured with LS55 fluorescence spectrofluorometer (Perkin–Elmer) at the wavelength of 450 nm. The fluorescence intensity of the luminescent materials was normalized and plotted as the mean values of three measurements ± SD (standard deviation).