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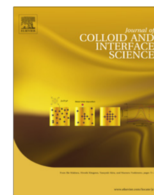

A simple one-step method for preparation of fluorescent carbon nanospheres and the potential application in cell organelles imaging

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Highly fluorescent carbon nanospheres with a quantum yield of 17.6% have been prepared by a one-step method with hydrothermal treatment of spider silk. Due to the high photostability, low toxicity and well blood compatibility, these carbon nanospheres could be used as an excellent probes for cancer cell imaging.

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1. Introduction

Fluorescent nanodots have attracted much attention owing to their unique fluorescent properties that possess wide application potential in biological systems, such as tumor cells detection and tracking. However, the application is shadowed by the latent toxicity of these nanodots because of the heavy metals in the nanodots. Carbon based nitrogen-doped nanospheres (CNP) would be a promising alternative on account of the unique properties, such as low photobleaching, no optical blinking, low toxicity and well biocompatibility, which made CNP favorable in its application in biological systems that are superior to traditional organic dyes and quantum dots [1–7].

Currently, most reports were focused on how to develop CNP with excellent characters through different ways [3,8]. Among all these methods, biomaterials based one step method is used due to the ease of obtaining and non-toxicity, such as cocoon silk, chitosan and orange juice [2,9,10]. Spider silk is a kind of protein fiber spun and used by spiders for the prey capture, prey immobilization, reproduction and other functions. Spider silk possesses many distinct properties, including strong strength, high density, high toughness, etc. [11]. Herein, we reported a one-step hydrothermal method to obtain water soluble CNP that utilized spider silk as the only material. After 72 h incubation, some yellow liquid was obtained with a size of 178 nm. There were many oxygen and nitrogen functional groups on the surface of CNP, which were characterized by FTIR and X-ray photoelectron spectroscopy (XPS). The Fluorescent property of CNP was carefully evaluated and there was an emission peak at about 500 nm with a quantum yield (QY) of ca. 17.6%. Moreover, the intracellular behavior of photoluminescent CNP was examined with organelles labeling. To address the safety concerns in the biological application, toxicity of CNP was evaluated by both cytotoxicity and hemolysis.

2. Materials and methods

2.1. Materials

DAPI and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Beyotime (Haimen, China). LysoTracker Red and MItoTracker Red were purchased from Life Technologies (Grand Island, NY, USA). Plastic cell culture dishes and plates were obtained from Wuxi NEST Biotechnology Co., Ltd. (Wuxi, China). Dulbecco's Modified Eagle Medium (high glucose) cell culture medium (DMEM) and FBS were obtained from Life Technologies (Grand Island, NY, USA). U87 cell line was obtained from Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China).

2.2. Preparation and characterization of CNP

CNP could be obtained from directly hydrothermal treatment of about 100 mg of spiker silk in 10 mL of deionized water at 200 °C

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for 72 h. The hydrated diameter and zeta potential were determined by a Malvern Zetasizer (Malvern, NanoZS, UK). The morphology was captured by transmission electronic microscopy (TEM) (JEM 100CX, JEOL, Japan). Fluorescence spectroscopy was evaluated by a Shimadzu RF-5301PC spectrofluorophotometer. UV–Vis spectra dispersed in water were performed on a Varian cary 100 conc UV–Vis spectrophotometer. Fourier transform infrared (FTIR) spectra were carried out on a Bruker Vector22 spectrometer (Germany) using spectroscopic grade KBr. X-ray photoelectron spectroscopy (XPS) experiments were performed on a AXIS Ultra DLD (Kratos UK) with Mg Ka radiation ($h\nu$ = 1486.6 eV), with a chamber pressure of 2.2×10^{-9} Torr. The source power and high voltage was set at 150 W and 15 kV and pass energies of 40 eV for survey scans were used. The analysis spot size was 300×700 μm. The data was analyzed by PHI-MATLAB software with C1s = 284.6 eV as a benchmark for the binding energy correction. Fluorescence quantum yields were evaluated using a RF-5301PC spectrofluorophotometer (Shimadzu, Japan).

2.3. Stability of CNP

The stability of CNP was evaluated in PBS with different concentrations of FBS. CNP were suspended in 0%, 10%, 50% or 90% FBS and incubated in a 37 °C incubator. The absorption at 570 nm were determined by a microplate reader (Multiskan MK3, Thermo, USA) at 0, 1, 2, 3, 4, 6, 8, 12 and 24 h.

2.4. Hemocompatibility

Whole blood was collected from mice using heparin as anticoagulant. After centrifugation at 1500 rpm for 5 min, the red blood cells were resuspended in PBS at the final density of 2%. Different concentrations of CNP were added into cell suspension and incubated at 37 °C for 60 min. The adsorption at 560 nm was determined by a microplate reader (Multiskan MK3, Thermo, USA). PBS was used as negative control while 1% of Triton X-100 was used as positive control.

2.5. Cytotoxicity of CNP

U87 cells were seeded into a 96-well plate at a density of 2×10^4 cells/mL. After 24 h, each well was added with CNP at a serial of concentration and incubated for another 24 h. Then 20 μL of MTT solution (5 mg/mL in PBS) was added into each well and cells were further incubated for 4 h under 37 °C. Then cells were dissolved by 150 μL dimethyl sulfoxide after removing the media. The absorbance was measured by a microplate reader (Thermo Scientific Varioskan Flash, USA) at 490 nm.

2.6. Cellular uptake

U87 cells, a human glioma cell line, were seeded into cover slip-contained plate at a density of 1×10^5 cells/mL. After 2 days incubation, cells were treated with different concentrations of CNP in FBS-free DMEM for 1 h. After nuclei were stained by 0.5 μg/mL DAPI for 5 min, fluorescent distribution in cells were observed by a confocal microscope (LSM710, Carl Zeiss, Germany).

2.7. Subcellular localization

After U87 Cells in plates incubated with 100 μg/mL of CNP for 0.5 h, LysoTracker Red or MitoTracker Red were added into wells at a final concentration of 100 nM and incubated for another 0.5 h. Nuclei of cells were stained and the cells were imaged as described above.

3. Result and discussion

3.1. Characterization of CNP

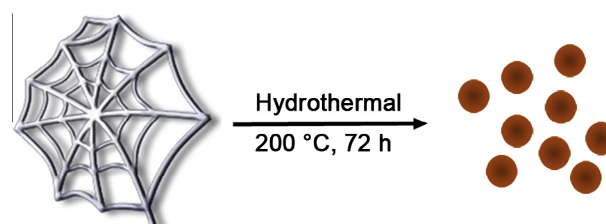
Hydrothermal treatment of spider silk at 200 °C for 72 h resulted in a yellow dispersion (Scheme 1). Dynamic light scattering (DLS) showed the hydrated particle size of CNP was 178 nm with a polydispersity index of 0.101. Transmission electron microscope showed CNP were spherical with uniform size and well dispersed (Fig. 1a). Zeta potential of CNP was −5.2 mV, which was mainly due to the carboxyl units in the surface of CNP and was useful for remaining stable in aqueous solution. In the FTIR analysis of CNP (Fig. 1b), the following peaks were observed: stretching vibrations of C—OH at 3424 cm^{-1}, asymmetric stretching vibrations of C—NH—C at 1115 cm^{-1}, stretching vibrations of C═C at 1598, 1513 and 1456 cm^{-1}, suggesting the structures of CNP might contain polycyclic aromatic and aromatic CN groups [2,8,12]. Surface states of the CNP were characterized by XPS. The XPS spectrum showed three peaks at 284.6, 399.7, 531.5 eV (Fig. 1c), contributed by C_{1s}, N_{1s}, and O_{1s} respectively. The C_{1s} spectrum (Fig. 1d) displayed four peaks at 284.6, 285.8, 286.2, and 288.0 eV, which were attributed to C—C, C—N, C—O, and C═N/C═O, respectively [13]. The N_{1s} spectrum (Fig. 1e) showed three peaks at 399.5, 400.4, and 401.5 eV, contributed by the C—N—C, N—C, and N—H bonds, respectively [14]. The O_{1s} spectrum (Fig. 1f) exhibited two peaks at 531.2 and 532.2 eV, which were attributed to C═O and C—OH/C—O—C groups, respectively [2]. These results demonstrated the surface of CNP possessed plentiful oxygen and nitrogen functional groups, which were useful for further modification.

Fig. 2a showed the optical properties of the CNP. There was obvious adsorption peaks at 208 nm, which was the typical properties of fluorescent carbon dots. When excited at 370 nm, CNP displayed strong blue photoluminescence with a peak at 447 nm. In the room temperature, CNP showed a clear yellow state and could be excited into strong color blue under UV light (365 nm, Fig. 2b). The fluorescent properties enabled CNP with the potential application in bioimaging, such as cancer cells detecting.

3.2. Cellular uptake

Although some studies showed CNP could be taken up by cells, the interaction of CNP with biological systems was far from well-known [2,10,15]. This knowledge is crucial for the application of CNP in biological systems, especially for the tracking/imaging of diseased cells in vivo. In this study, U87 cells, a human glioblastoma cell line, was used to evaluate the behavior of CNP.

It was showed CNP could effectively be taken up by U87 cells (Fig. 3a). Increasing concentration of CNP could enhance the cellular uptake by U87 cells, suggesting the uptake was in concentration-dependent manner and it was not saturated at a concentration as high as 500 μg/mL. Normally, particles were internalized into cells through an endosome-mediated pathway. However, CNP showed few colocalization with endosomes labeled



Scheme 1. Illustration of preparation procedure of CNP by hydrothermal treatment of spider silk.

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